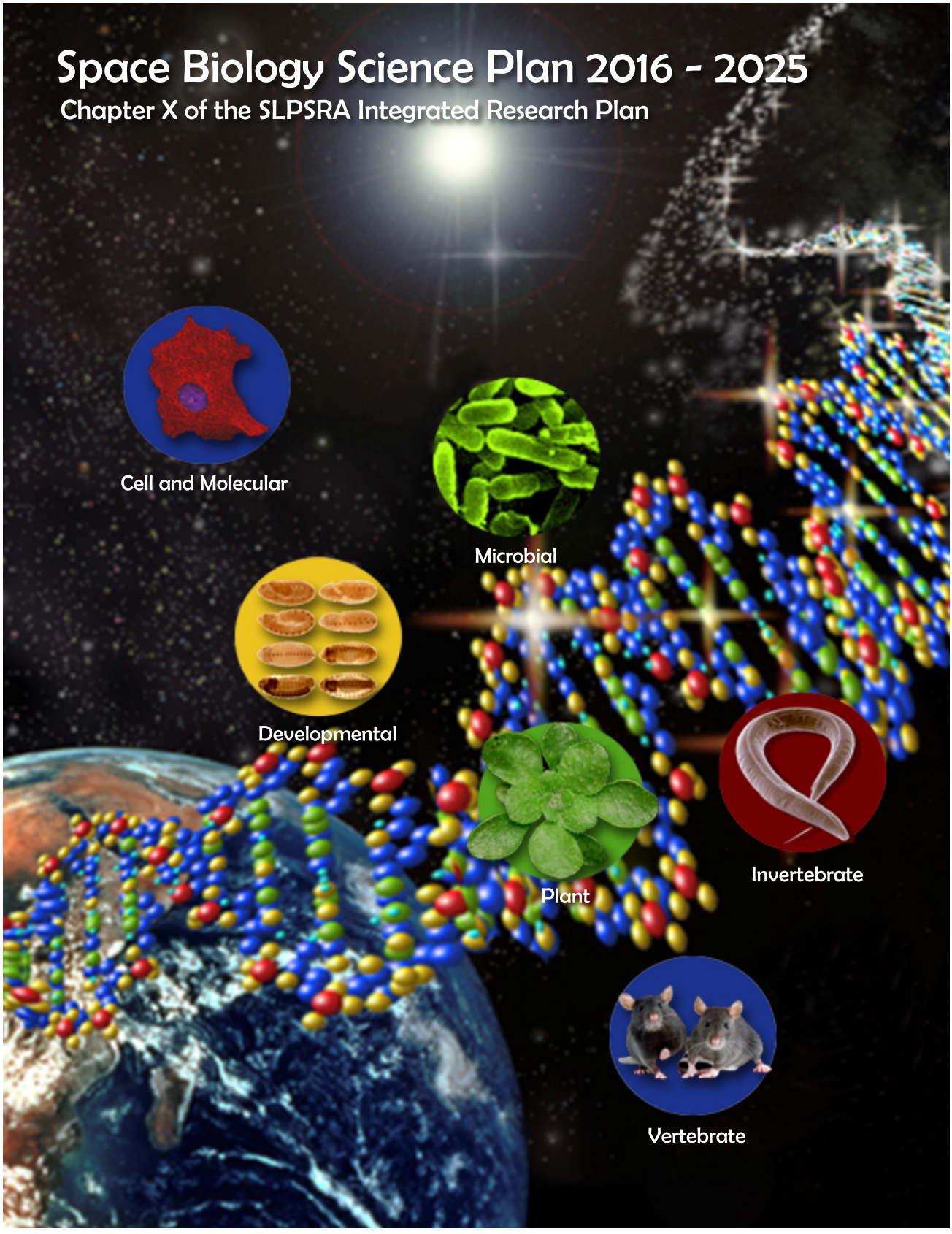
Space Biology Science Plan 2016 - 2025
Chapter X of the SLPSRA Integrated Research Plan
Cell and Molecular
Microbial
Developmental
Plant
Invertebrate
Vertebrate

Dedication

A core team of authors began working on this plan for Space Biology research in mid-June 2015 at a retreat at Ames Research Center. The team that assembled at Ames that week included David Tomko, Ken Souza, Jeff Smith, Richard Mains, Kevin Sato, Howard Levine, Charlie Quincy, Aaron Mills and Amir Zeituni. Although others have contributed to our efforts over the past year, this team of authors bears principal responsibility for its shape and content. During the past month Ken worked with me almost non-stop on the final polish, to insure as much as humanly possible that errors were eliminated and nothing was left out. His contribution to Space Biology is immeasurable, the Plan would not exist in its present form without his work, and we will sorely miss working with him in the future. It is therefore with the greatest respect and admiration that we dedicate this Space Biology Plan to the memory of our dear friend, colleague and mentor Ken Souza, who worked tirelessly, well and hard with us on its genesis. – The Authors, March 23, 2016

Chapter X In SLPSRA Integrated Research Plan

Space Biology Plan

2016-2025

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I. Background

Introduction. In 2010, NASA published a "NASA Fundamental Space Biology (FSB) Science Plan, 2010-2020" to guide its research investments in this area of Space Life Sciences. The Space Biology goals set by NASA for the decade 2010-2020 included:

1) Sponsor competitively solicited FSB research to create new knowledge of how biological systems adapt to space and that can translate into Earth benefits;
2) Use ISS, free-flyer, ground-based analogues or other venues to conduct cutting-edge FSB research;
3) Maintain an internationally competitive United States FSB scientific community;
4) Develop cutting edge technologies to facilitate conduct of biological research in spaceflight;
5) Issue regular NRA's to reengage the U.S. Space Biology community;
6) Work with international partners and other U.S. agencies to achieve objectives; and
7) Train and inspire the next generation of U.S. Space Biologists.

The priorities were outlined in the following table:

Priority	2011-2015		Priority	2016-2020
High	Cell, Microbial and Molecular Biology on ISS		High	Animal and Plant research on ISS
	Development of Plant and Animal Habitats			Cell, Microbial and Molecular Biology on ISS
	Expanded Ground Res.: Plants, Animals, Cells			Free Flyers: Bion-M3
	Free Flyers: Bion-1, Bion-M2			Microsatellites
Medium	Microsatellites		Medium	Ground & Flt Research - Developmental Biology
	Advanced Technologies for ISS and Free Flyers			Ground Research - Plants, Animals, Cells
	Ground Research - Developmental Biology			Advanced Technologies for ISS and Free Flyers
	Education and Outreach			Education and Outreach
Low	Flight Research - Developmental biology		Low	Sub-Orbital Research

In brief, 2011-2015 was to be devoted to:

1) pursuing flight research in areas that were already strong, microbiology, cell and molecular biology as well as building a more robust ground-based program;
2) completing planned free-flyer experiments; and
3) building new flight experiment hardware capabilities in cell and molecular, animal, and large plant biology.

Space Biology has made investments to accomplish these 3 objectives. Already strong ground-based and flight research areas were strengthened. A principal free-flyer experiment, Bion M1 was completed, with nine collaborative U.S./Russian experiments flown. New flight hardware to enhance NASA's ability to fulfill recommendations of the NRC's Decadal Survey[1] in all areas of Space Biology have been completed, or are nearing completion. Fulfilling the goals of the second half of the decade requires continued access to flight opportunities and increasing the scope of ground-based activities.

Congressional Direction to NASA. In May 2009, the National Research Council's (NRC) Committee for the Decadal Survey on Biological and Physical Sciences in Space began a series of meetings initiated as a result of the following language in the explanatory statement accompanying the FY 2008 Omnibus Appropriations Act (P.L. 110-161):

[1] "Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era" http://www.nap.edu/catalog/13048/recapturing-a-future-for-space-exploration-life-and-physical-sciences

"Achieving the goals of the Exploration Initiative will require a greater understanding of life and physical sciences phenomena in microgravity as well as in the partial gravity environments of the Moon and Mars. Therefore, the Administrator is directed to enter into an arrangement with the National Research Council to conduct a "decadal survey" of life and physical sciences research in microgravity and partial gravity to establish priorities for research for the 2010-2020 decade."

In response to this direction, NASA commissioned an NRC study in consultation with members of the life and physical sciences communities, NASA, and congressional staff. The guiding principle of the study was to set an agenda for research in the next decade that would use the unique characteristics of the space environment to address complex problems in the life and physical sciences, so as to deliver both new knowledge and practical benefits for humankind as it embarks on a new era of space exploration. Specifically, the decadal survey committee was asked to define research areas, recommend a research portfolio and a timeline for conducting that research, identify facility and platform requirements as appropriate, provide rationales for suggested program elements, define dependencies among research objectives, identify terrestrial benefits, and specify whether the results of the research would directly enable exploration or would produce fundamental new knowledge.

Major NRC Decadal Survey Recommendations. In 2011, the Committee for the Decadal Survey on Biological and Physical Sciences in Space of the National Research Council published its decadal survey recommendations to NASA, "Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era" that established guidelines for NASA's approach to conducting research in the Space Life Sciences. Major recommendations of the Decadal Survey included to conduct:

1) Systematic research studies that use ISS as a Microbial Observatory;
2) Cell and Molecular Biology studies using state-of-the-art cell biology tools to monitor evolution of genomic changes in microbes, plants, animals or other biological systems in spaceflight;
3) A systematic suite of plant biology experiments to elucidate mechanisms by which plants respond and adapt to spaceflight, and to facilitate their eventual use in Bioregenerative Life Support Systems;
4) Animal and human studies to evaluate physiological mechanisms of bone, muscle, cardiopulmonary, immune, and neural functions during adaptation to spaceflight;
5) Studies that will determine how spaceflight affects reproductive, developmental, and evolutionary mechanisms;
6) Cross-cutting studies, including artificial/fractional gravity, radiation, and gender differences; and
7) Activities facilitating open public and scientist access to the products of NASA Space Biology research data and results by building data archives and data management tools, especially in the area of systems biology (genomic and other "omic" experiments).

II. Summary of Progress since the 2010 Space Biology Science Plan.

Goals of the 2010 Space Biology Science Plan and summary of progress. The purpose of this update to the 2010 Space Biology Plan is to review progress against the 7 goals, and to specify the goals and approaches for the next 10 years.

Goal 1) Sponsor competitively solicited FSB research to create new knowledge of how biological systems adapt to space and that can translate into Earth benefits; since 2010, 55 flight and 28 ground-based Space Biology projects were funded, resulting in more than 350 peer reviewed publications.

Goal 2) Use ISS, free-flyer, ground-based analogues or other venues to conduct cutting-edge FSB research; Space Biology has solicited research across all the areas of science recommended by the Decadal Survey. During the time between 2010 and 2015, 88 extramural research projects were funded in Plant and Animal Biology, Cell and Molecular Biology, and Microbiology. Eleven Space Biology experiments were flown on the ISS with the other 44 flown on the combination of the Space Shuttle, Bion M1, and microsatellites..

Goal 3) Maintain an internationally competitive United States FSB scientific community; Space Biology has continued the long tradition of adding significant peer-reviewed contributions to the primary literature, as shown in Figure 1 below.

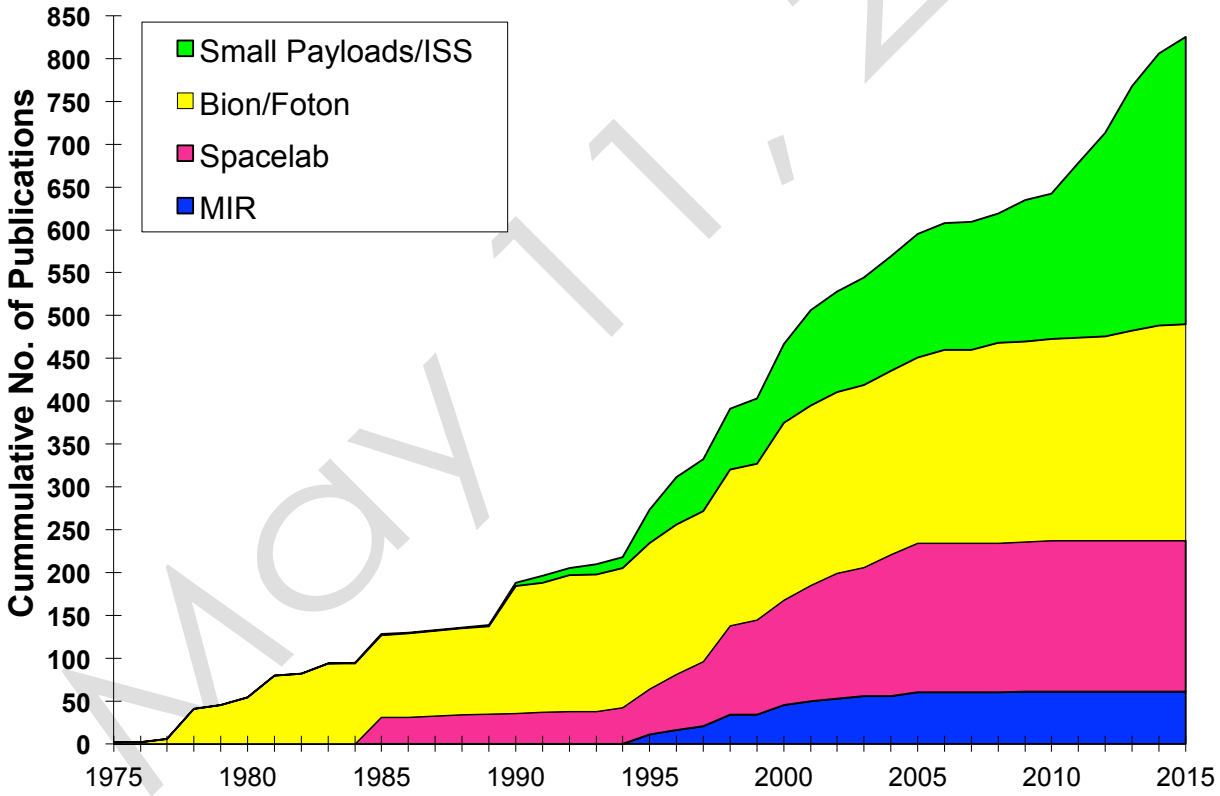

Figure 1 – NASA Space Biology Research Publications 1974-2015 shows the steady accumulation of new knowledge over the past 40 years of the Space Program.

Goal 4) Develop cutting edge technologies to facilitate conduct of biological research in spaceflight; Consistent with the 2010 plan, Space Biology has led the development and execution of a wide variety of new capabilities for ISS, including a Rodent Research Facility, the VEGGIE plant growth chamber, the Advanced Plant Habitat, the BioCulture System for Cell Biology Research, a

Wetlab Facility for on-orbit qRT-PCR analyses, and a drosophila lab. In addition, critical database infrastructure support for state-of-the-art multiomic studies in Space Life Sciences is under development to enable the scientific, commercial and general community to have ready access to these data and tools for studying them, and developing new testable spaceflight hypotheses.

Goal 5) Issue regular NASA Research Announcements (NRA's) to reengage the U.S. Space Biology community; since 2010, NASA SLPS has issued 4 flight experiment, 2 ground-based, and 2 biospecimen-sharing NRA's, as well as a single New Investigator NRA. A total of 272 proposals were received across all the Space Biology elements, and a total of 81 proposals were funded. Across the last 2 NRA's there were 35 investigations selected, of which 23 were led by investigators new to Space Biology.

Goal 6) Work with international partners and other U.S. agencies to achieve objectives; There have been 2 International Life Sciences Research Announcements soliciting research proposals for ISS, one in 2009 and a second in 2014. These solicitations were coordinated with ISS partners, ESA, CNES, DLR, JAXA, and CSA. Forty-nine NASA Space Biology proposals were received in response to these 2 solicitations, and 16 research projects were funded based on the peer-reviewed results, and technical feasibility assessments. In addition, NASA SLPS collaborated with the Russian Space Agency and the Russian Institute for Biomedical Problems on the 2013 Bion M1 flight, supporting 8 NASA investigations. Discussions of future opportunities are underway. Both the International Space Life Sciences Working Group (ISLSWG) and Russian cooperation led to joint investigator studies.

Goal 7) Train and inspire the next generation of U.S. Space Biologists. Since 2010, NASA Space Biology funds 36 undergraduate, 48 graduate and 31 post-doctoral fellows through individual investigator research grants. In addition, Space Biology has supported the NASA Post-Doctoral Program for apprentice scientists who wish to train as post-docs in NASA Center Laboratories. Since 2010, 10 NPP fellows have been funded by Space Biology for post-docs working in NASA center laboratories.

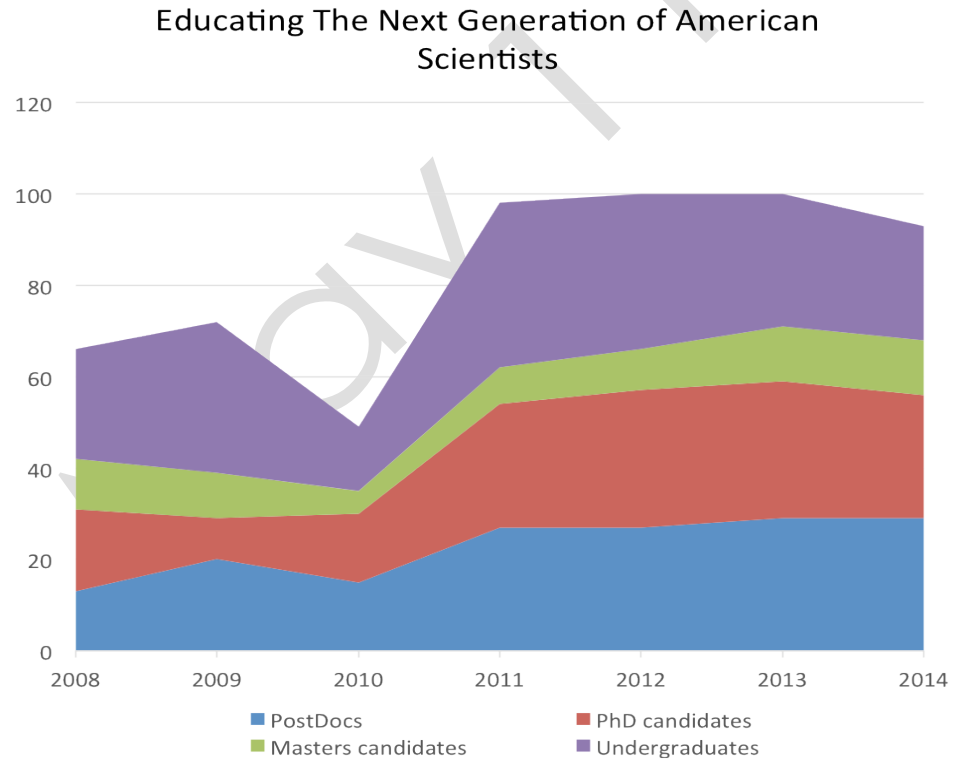

Figure 2 – Currently funded students and post-doctoral fellows working on ISS Space Biology flight experiments

III. Space Biology Plans 2016-2025

A. Space Biology Overview

Introduction. For an estimated 3.8 billion years, the evolving microorganisms, plants, and animals on Earth lived and diversified in a consistent physical context of gravity, partially shielded from the magnetic fields and radiation of outer space. Trends toward more complex environmental factors, particularly the effects of other species, have exerted further pressures on the genetic complement of Earth's life forms. Currently, with an almost 4 billion year history of exposure to the Earth's environment, terrestrial life is well adapted to its home planet. The future holds the prospect of a sudden and rapid change for many Earth bound life forms—the prospect of extended travels beyond their planet of origin.

At the beginning of our ventures into space, the hazards and risks of spaceflight were unknown. Some feared that in the absence of gravity the heart would not pump properly, problems in digestion would occur, and radiation in low Earth orbit (LEO) would cause mutations and increase cancer risk. Animals and a variety of other organisms were used during the early years of spaceflight to evaluate such risks and demonstrate that spaceflight was safe for human exploration. In addition, biologists began using the unique environment of space as a tool to understand how life adapts to changes in gravity and how it responds to the hazards of space radiation. During the past 50+ years, space biologists have been able to identify and clarify many of the effects of spaceflight on representative living systems, from the cellular, tissue and system levels, to the whole organism. We've learned much about how life reacts to the challenges of spaceflight and the space environment, but many of the mechanisms living systems use to sense and react to such challenges remain unknown. Knowledge of underlying biological mechanisms enables better informed decisions on the risks of spaceflight to humans, and the design of countermeasures to those risks.

Space Biology (SB) has been part of NASA's Life Sciences Program from the 1960's to the present. Today's program increasingly focuses on using the rapidly evolving technologies of cell and molecular biology to answer basic questions about the effects of spaceflight on biological processes. SB funds research studies that will determine how changes associated with space travel will affect a diverse group of microorganisms, plants, and animals. Research focuses on the effects of gravity from hypo- to hyper-gravity, as well as biological effects of space radiation, altered magnetic fields, and interaction of species in space and spacecraft.

Throughout the course of evolution, gravity has greatly influenced the morphology, physiology, and behavior of life. For example, the musculoskeletal system evolved to support body mass as aquatic animals transitioned to land. Similarly, the colonization of land by upright plants was facilitated by the production of structural reinforcement compounds such as lignin. Spaceflight experiments, capitalizing on the microgravity environment to shed light on the production of these compounds, have found that although lignin biosynthesis is decreased during spaceflight (Cowles, et al., 1994: PMID 11540197; Sato, et al., 1999: PMID 11542476), it is still produced in sufficient quantities to provide requisite mechanical stress responses (Kwon, et al., 2001: PMID 11423136).

To orient and ambulate in their environment, organisms developed ways to sense gravity and translate this information into a controlled response; hence the sensorimotor system evolved in animals, and analogous gravity sensing mechanisms evolved in plants. A robust cardiovascular system developed to maintain an appropriate blood supply and pressure in the various organs of the mammalian body. The development of the phloem and xylem vasculature in plants mirrors this development. Understanding how these physiological systems sense, adapt and respond to gravity cannot be fully achieved on the ground; it requires the use of spaceflight, i.e., the use of microgravity as an investigative tool. Just as one needs to examine the entire light spectrum in order to determine the capabilities and mechanisms of the visual organs, so too must we utilize the complete gravity spectrum, from hypo-gravity to hyper-gravity, to understand how gravity influences life across the gravity continuum, i.e., both on and off the Earth.

Space Life Sciences spans the biological continuum from molecular to human clinical research, as shown in Figure 3. Space Biology researchers use scientifically characterized microbes, plants, insects and animals to understand how organisms, including humans, respond to the space environment. NASA's Human Research Program (HRP) covers the clinical and operational research on the opposite end of the continuum, and uses knowledge gained about biological adaptation to spaceflight in practical application. Utilizing the new molecular biology tools of the 21st century, Space Biologists probe deeply into underlying mechanisms of adaptation to the space environment in order to determine the fundamental ways life uses gravity to regulate and sustain its growth, metabolism, reproduction and development, and also how it repairs damage and protects itself from infection and disease. Such basic knowledge provides the foundation on which NASA's biomedical researchers in HRP build approaches and countermeasures to the problems confronting human exploration of space. In addition, such knowledge has provided, and will continue to provide, benefits to the health and well-being of those on Earth. NASA SLPS has made deliberate efforts to foster collaboration/cooperation between SB and the HRP ("NASA Translational Research Roadmap (TRR) Workshop, Co-chairs, W. Paloski, NASA JSC; D. Tomko, NASA HQ, October 23, 2014, held at the American Society for Gravitational Space Research Conference, Pasadena, CA, online at: https://ac.arc.nasa.gov/p1lf9sla9od?launcher=false&fcsContent=true&pbMode=normal; and Ronca, A.E., K.A. Souza and R.C. Mains, "Translational Cell and Animal Research in Space, NASA Ames Research Center, 1965 - 2011. NASA/SP-2015-625 Government Printing Office)

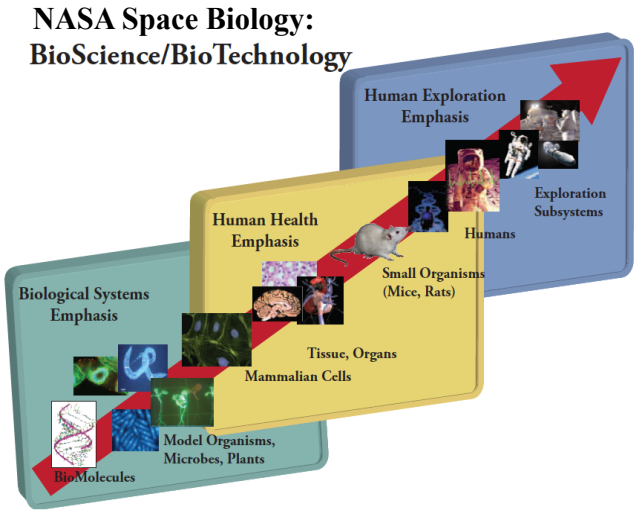

Figure 3 – NASA Space Biology occupies a unique niche in the Space Life Sciences continuum. It sponsors research to improve our understanding of basic biological processes along the continuum in this figure. Space Biology research provides basic findings that are "deliverables" to be used in producing translational products for human applied and clinical problems (e.g., to the NASA HRP) and to potential commercial users (e.g., the Center for the Advancement of Science in Space, CASIS).

The Next 10 Years. Microbial, animal, and plant research will be emphasized during the next ten-year period (2016-2025). Space Biology will use biotechnology advances, including the ever-increasing availability of genetically engineered organisms to understand the molecular mechanisms that microbes, animals and plants use to respond to gravity transitions. Long duration studies with animals, e.g. 3-6 months or more, will determine how the musculoskeletal system changes over time in microgravity, as well as the impact of long duration flights on the immune system. Multiple generations of microbes and plants will be studied both to understand the long-term effects of microgravity and to demonstrate the potential use and reliability of biological components for future advanced life support systems. It is anticipated that cooperative projects with our international partners will continue and will include research both on the ISS and free flyers. In addition, ad-

vanced technologies will continue to be applied to bring more capability to microsatellite missions, including live return of organisms.

Ground-based Research leads to Space Experiments. Research on the ground has always been the basis for developing and refining hypotheses to be tested in space. Proven analogs such as rotating wall vessels or rodent hind limb unloading that induce biological effects similar to spaceflight will be used in studies of model organisms to focus on critical overarching questions. Research will be solicited and selected transparently after scientific peer review, to insure that the best and brightest scientists are performing state-of-the-art science, and researchers will be held accountable for public dissemination of the results of their federally-funded research. To insure the development of the best hypotheses that need to be developed and tested in spaceflight, it is anticipated that a ratio of approximately 3:1 (ground: flight studies) should be maintained for overall program balance and cost effectiveness.

GeneLab. To facilitate open access to spaceflight data acquired by investigations on ISS, other spaceflight platforms, or ground-based simulation or centrifugation experiments, NASA Space Biology has implemented an "open-science" approach, which includes the development of a database and specimen repository of all Space Life Sciences flight data, most importantly, those data acquired in experiments seeking genomic and other "omic" data. It is NASA's intention that these data and the knowledge stored in this database will be used by researchers to develop new hypotheses to be tested on the ground or in spaceflight, in amplifying the results of other studies, or in the development of commercial products or other translational tools. The overall GeneLab initiative is presented in figure 4 below.

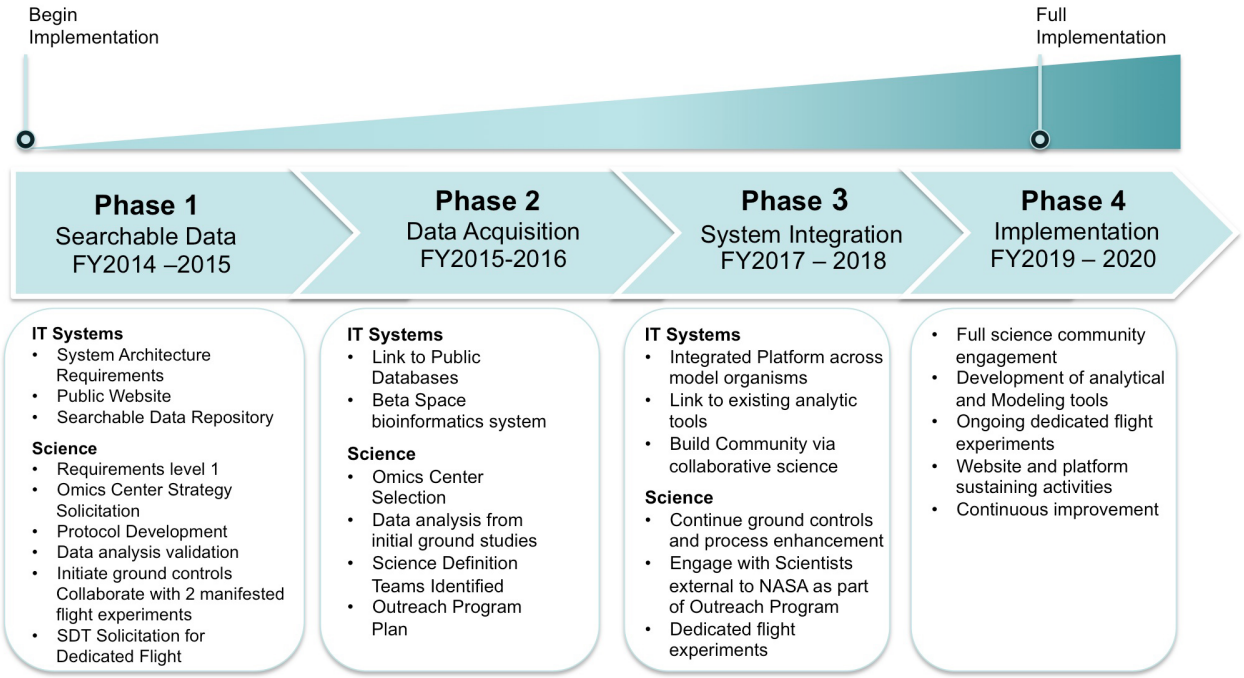

Figure 4 – The plan for implementing the GeneLab Project which will build the infrastructure for Open Science access to spaceflight systems biology "omics" data collected by scientists from ISS and other platforms. The GeneLab database will enable researchers to develop new hypotheses from archived data.

Technology development will insure state-of-the-art NASA research products. New areas of research may require new equipment and/or technologies. The SB Program will conduct regular assessments of its research needs via workshops and reports from appropriate advisory committees and/or organizations. Development of high priority equipment or technologies will be prioritized by the FSB Program and developed according to applicable NASA policies and practices. Miniatur-

ized, highly sensitive measuring devices lead the latest wave of new tools destined to equip Space Biology projects for spaceflight. These devices require cell, microbial, plant and animal model systems that will rapidly return high quality data from flight experiments, e.g. imaging, environmental, biochemical, animal health and physiology, etc. Computing capacities must accelerate to keep pace with the expanding needs for processing and analyzing the data gathered from these experiments. The SB Program will also continue its efforts to improve the frequency of access to space, e.g., enhancing the availability and capabilities of microsatellites.

"Gravity as a Continuum Project" in Space Biology. As NASA contemplates long duration human spaceflights beyond low Earth orbit, research across the gravity spectrum assumes an increased importance. The Space Biology Program is initiating a new project to address this need systematically. It is called the "Gravity as a Continuum Project". All Space Biology projects must manipulate gravity as an independent variable, a continuum, from micro-gravity to hyper-gravity. Both basic research and development of astronaut countermeasures require knowledge from experiments performed in or on:

1) Facilities in space to allow low vibration micro-gravity exposure during spaceflight to organisms ranging in size from cell cultures to humans, similar in concept to the planned rotating Mars Gravity Biosatellite (Wagner and Fulford-Jones, 2006: PMID 16630591);
2) Spacecraft centrifuges that can produce fractional gravity levels between micro-gravity and Earth gravity as well as hypergravity. The results of past studies using such centrifuges indicate that partial levels of gravity ranging from 0.3g-0.5g are enough to prevent some of the biological effects of microgravity in plant seedlings and human lymphocytes. (Kiss et al., 2012: PMID 22481136; Kiss, 2014: PMID 23889757; Hughes-Fulford et al., 2015: PMID 26276131);
3) Ground-based centrifuges that produce hyper-gravity levels from Earth gravity to 3-5 times Earth gravity, which have successfully been used in past studies to assess the effects of hypergravity on rodent models. Such studies have helped identify inverse relationships between gravity and body mass, as well as between gravity and metabolic activity (Wade, 2005: PMID 16101110; (Plaut et al., 2003: PMID 12923115; Oyama and Platt, 1967: PMID 6015997; Oyama and Platt, 1965: PMID 5891216);
4) Ground-based simulators such as High-Aspect Ratio Rotating Vessels or random positioning machines to mimic spaceflight effects on cell cultures and small animals and plants. Recent work with cultured mammalian lymphocytes has verified the ability of these simulators to induce changes in gene expression patterns that are similar to those induced by spaceflight (Martinez et al., 2015: PMID 25568077);
5) Ground-based unloading laboratory paradigms that enable small mammals to experience fractional unloading to simulate the partial Earth gravity of the moon or Mars. Past studies using this experimental system with rodents have demonstrated a direct relationship between the degree of unloading and the changes observed in bone density and architecture, and muscle tissue. Such results validate the use fractional unloading as a surrogate model to study gravity as a spectrum (Wagner et al., 2010: PMID 20522735; Ellman et al., 2013: PMID 23165526).
6) Parabolic flight opportunities where a wide range of gravity levels between microgravity and 2 times Earth gravity are available for short periods of time. Use of this model to simulate lunar gravity has revealed that gravitational strength influences the behavior of aerosol particles in the human lung (Darquenne and Prisk. 2008: PMID 18488244).

It is NASA's intent to facilitate Space Biology experiments in this area from cell biology up to mammalian organisms by soliciting research projects that will systematically manipulate this important independent variable to understand the impact of microgravity as well as fractional gravity on biological systems. Experiments will be designed in particular to understand responses of biological systems to lunar and Mars gravity.

B. GeneLab, Systems Biology, Omics & Open Science in Space Biology

Introduction and background. This approach has been pursued in an organized way since 2013. NASA Space Biology has sought the recommendations of Life Sciences experts to help stand up a GeneLab database. A GeneLab steering committee helped identify key pitfalls and made recommendations to ensure the type of positive exposure for the database within the greater life science community. In September 2014 a GeneLab Strategic Plan was baselined and published (http://genelab.nasa.gov/resources/GeneLabStrategicPlan_Baseline_2014.pdf). On April 15, 2015 the first phase of the GeneLab database was online and operational https://c3.nasa.gov/genelab/). On April 24, 2015 GeneLab received a "Webby award" in the Websites: Best Navigation category. The GeneLab Project staff has been actively engaging with currently funded Space Biology investigators and in some cases have partnered with PI's to increase sample biomass for additional omics analysis in what is referred to as "GeneLab piggyback" augmentations. Space Biology has augmented formally two investigator grants (Gilroy, Wyatt) to fly extra specimens to increase biomass for analysis and inclusion in the GeneLab database. Other genomic studies in the Space Biology portfolio are being included based on the investigator's proposed experiment only.

GeneLab staff is currently searching NCBI, GEO, EBI and other large databases for NASA Space Biology legacy data, as well as Space Biology data from other NASA partners. When a project containing space-based data is identified, it is incorporated into the GeneLab database, and appropriate ISS or other platform metadata fields are updated and incorporated. This ensures that all known NASA Spaceflight experiments and appropriate ground analogs and research tools (radiation, centrifuge, HARV, HLU, bedrest, etc) are in a centralized repository. To date 55 "legacy" data sets have been included in the GeneLab database.

Goals are stated in the GeneLab Strategic Plan at http://genelab.nasa.gov/discovery-genelab-strategic-plan.html They are as follows:

- GeneLab will gather spaceflight genomic data, RNA and protein expression, and metabolic profiles, interface with existing databases for expanded research.
- Develop an integrated repository and bioinformatics system for analysis and modeling
- Enable the discovery and validation of molecular networks that are influenced by space conditions through ground-based and flight research using next generation omics technologies
- Engage the broadest possible community of researchers, industry and the general public to foster innovation
- Strengthen international partnerships by leveraging existing capabilities and data sharing
- Early studies are using Commercial Generic Bioprocessing Apparatus (CGBA) and Biological Research in Canisters (BRICs)

The objectives are as stated in the GeneLab Strategic Plan:

- Increase opportunities for multi-investigator spaceflight opportunities
- Democratize Space Biology research by integration of various types of bioinformatics analytics such as genomic, transcriptomic, metabolomic and proteomic data into a publically available and searchable database
- Translate GeneLab data into information that will increase the knowledge and discovery output from what would have traditionally been a single PI opportunity
- Facilitate and encourage community-driven reference experiments to generate standard, common, and open reference datasets to act as a powerful resource for scientific throughput and innovation.

The following Guiding Questions for research conduct in this area were developed from Decadal Survey embedded recommendations:

GL1 – Can robust transcriptional proteomic and metabolic profiling of responses of microbes, plants and animals exposed to spaceflight provide a heuristic resource for development of new hypothe-

ses, new experiments, new understanding, and new publications? (Decadal Survey pages 59, 60 and 72)

GL2 – Can raw datasets, including unprocessed molecular data that can be subjected to subsequent analyses lead to increased use of ISS data to facilitate new science products? (Decadal Survey page 60).

GL3 – What are the key cellular and molecular systems necessary for biological organisms to thrive in space environment that can be discovered from 21st century bioinformatics tools? (Decadal Survey page 71).

GL4 – Can the application of modern analytical techniques such as those employed in genomics, transcriptomics, proteomics, and metabolomics improve our understanding of the effects of spaceflight by organizing a formalized program to promote the sharing and analysis where spaceflight data can be mined and analyzed by multiple researchers? (Decadal Survey pages 73).

GL5 – Can a program of analysis grants, dedicated to the analysis of spaceflight-derived datasets, provide value-added interpretation, while ensuring that all data are maximally mined for information? (Decadal Survey pages 73).

GL6 – Will large-scale, multiple-investigator experiments, with related science objectives, methods, and data products, result in the production of large datasets emphasizing replicates, statistical strength and modeling over implementation? (Decadal Survey pages 71 and 73).

Future Plans - Near term, GeneLab will develop the necessary framework and architecture to accept new omics data and to federate with the larger scientific community (e.g. with NCBI, EBI GEO and others).

Future NRA's - Space Biology is planning to conduct a community reference experiment in the CGBA or BRIC. NASA will form a Science Definition Team (SDT). An (SDT) is a team of Space Biologists who will advise NASA on how to conduct an experiment that will be of greatest value to the community. Investigators will work with NASA to define mission success criteria (e.g., weight of samples returned, duration of culture, types of omics data yielded), experimental conditions and range (e.g., temperature, volatile organic compounds, atmospheric composition, relative humidity, etc.), and appropriate omics centers to process the data. NASA will advertise the upcoming reference mission to the appropriate communities (American Society for Gravitational and Space Research, American Society for Microbiology, American Society for Cell Biology, American Society for Plant Biology, Federation of American Societies for Experimental Biology and others) and conduct the experiment with inputs from the Space Biology community. Data will be placed in the GeneLab Database. NASA will then issue a competitive ground based NRA for people to use the reference mission data to identify patterns and trends and to devise new testable hypotheses and experiments, potentially using appropriate gene knockouts and knockdowns or and other novel research tools.

NASA will issue a series of modest analysis grants dedicated to the analysis of spaceflight-derived datasets. These would provide value-added interpretation while ensuring that all data are maximally "mined" for information. NASA envisions targeting both Postdocs and graduate students with these types of analysis grants.

Genelab Technology Development. Phase III (see Figure 4) of the GeneLab project "Integrated Omics Capability" will initiate development of a platform capable of integrating various data types across model organisms. Space Biology plans to develop bioinformatics tools so that citizen scientists can integrate the GeneLab Database. Space Biology plans to devise a bioinformatics tool that can drill down between the various "omes" between different Domains and Kingdoms. This phase will expand the data system capabilities to include tools for data analysis, and maintain the data system as it grows.

- Technology and techniques for increased and improved data collection, data dissemination, deeper analyses, and data access within the GeneLab data system.

- Rapid freeze capability in spaceflight for "snap freezing" and for preserving proteins and metabolic products for ground omics analyses. To ensure minimal sample degradation, NASA must freeze (-80 C) its samples on orbit in a manner similar to how it is done on the ground.
- Continuous GeneLab data system improvements so that the data base remains current with continued advancements in omics and bioinformatics techniques and presentation/analysis methods.

Expected Outcomes. NASA envisions that GeneLab will act as a PI multiplier. NASA's plan of combining piggy-back experiments, SDTs, and ground based analysis NRA's to interrogate the database will ensure that ISS data will be analyzed and generate publications for years to come. The later phases of the GeneLab project aims to recapture the imagination of the American population. With their intuitive user interface and powerful bioinformatics software, GeneLab hopes to tap into the power of citizen science to foster new discoveries and novel hypothesis.

C. Space Biology Program Science Elements

For the purpose of this plan, NASA has divided Space Biology into 5 science elements based on its interpretation of the recommendations of the Decadal Survey – 1) Microbiology; 2) Cell and Molecular Biology; 3) Plant Biology; 4) Animal Biology; and 5) Reproductive, developmental and evolutionary Biology.

1) **Microbiology Element**

Goal. The overarching goal of the microbiology element is to determine the effect of spaceflight on microbial life, processes, and community dynamics.

Objectives included in this element are to:

a. Conduct long-term, multigenerational studies of microbes using the ISS as a Microbial Observatory to study and understand the population and community dynamics of the microbes that inhabit that unique environment.

b. Determine the influence of spaceflight on defined microbial populations and communities. Studies will determine the effects of spaceflight on dynamics of microbes in mono or mixed cultures with respect to cell processes (including virulence and antibiotic resistance, evolution, biofilm formation, and community development.

c. Simulate elements of spaceflight conditions such as microgravity in ground-based analogs such as clinostats, High Aspect Rotating Vessels (HARVs), or other Low-Shear Model-Microgravity (LSMM) systems, and parabolic flights. These studies will generate and test specific hypotheses to feed into the flight program as in b, above.

d. Develop approaches to examine interactions among microbes and other organisms such as plants and animals that effect important processes (e.g., commensalisms, symbioses, N-fixation, biodegradation and prevention of crew dysbiosis. Leverage opportunities with international partners and those of other federal agencies.

Guiding Questions and links to Decadal Survey Recommendations in the Microbiology Element include:

- MB-1 What underlying genetic, molecular and biochemical processes are influenced by the space environment? (P2)
- MB-2 What are the systems biology mechanisms and pathways for microbial life are responsive to spaceflight conditions? (P1, P2)
- MB-3 How does spaceflight influence microbial biofilm formation and microbial community development? (P1, P2, P3)

- MB-4 What are the mechanisms that effect changes such as the altered virulence or altered drug resistance observed in some organisms during spaceflight? (P1, P2, P3)
- MB-5 How does the spaceflight environment influence microbial reproduction, growth, and physiology? (P1, P2)
- MB-6 Does long -duration spaceflight alter normal rates of evolutionary change? (P1)
- MB-7 What is the effect of spaceflight on microbial communities as they interact with other organisms to effect processes such as symbioses, dysbiosis, biodegradation, nitrogen-fixation, etc. (P1, P2, P3)

Space Biology Microbiology History. Early studies with bacteria and fungi showed that they reached higher population densities when grown under microgravity conditions than were obtained from cultures grown under similar conditions on the ground. Higher cell density is likely due to a more homogeneous distribution of cells in the culture medium, as opposed to the crowded and more nutrient–depleted conditions as the cells settle that occurs at 1g. These studies also showed that spaceflight caused some bacterial species to become more resistant to common antibiotics (Klaus and Howard, 2006: PMID 16460819).

A ground breaking spaceflight experiment found that space- grown cultures of the pathogen *Salmonella typhimurium* were significantly more virulent than comparable cultures grown on the ground (Wilson, et al., 2007: PMID 17901201). RNA microarray analyses revealed changes in the gene expression of over 160 gene transcripts, one of which was a cross- species conserved RNA-binding regulator protein, Hfq, which is involved in RNA transcription and has been found to play a role in microbial virulence of several pathogenic bacteria. These data suggested that Hfq can play a critical regulatory role in the spaceflight response of bacteria and the observed increased virulence, a result that has profound implications for long duration spaceflight, and for understanding and potentially manipulating microbial virulence on Earth.

Space Biology Microbiology Progress since 2010. Since 2010, Space Biology has selected and funded 17 spaceflight experiments dedicated to microbiology. Some important examples include Venkateswaran's work, which represents first Space-Biology-funded examination of resident microbial populations on ISS. This study is generating a microbial census on ISS using state-of-the-art molecular biological tools, which directly addresses stated NRC recommendations. The investigators have already started to demonstrate mechanistic understanding of the changes that can occur in the space environment (Venkateswaran, et al., 2014: PMID 24695826). Data are available at: https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-26/ Future studies should examine how these communities expand, contract, and otherwise change over time.

Bacterial biofilms are an important and often problematic aspect of life on earth and in space. These biofilms are responsible for the biofouling and destruction of many materials, including those found on spacecraft, as well as being implicated in many infections. Biofilms under microgravity conditions have a higher level of resistance to antimicrobials, and therefore may represent a situation that is more difficult to treat in space. Collins' group demonstrated for the first time that microbes in biofilms form a unique column and canopy structure in microgravity (Kim, et al., 2013: PMID 23658630). These studies should be expanded to include other organisms and mixtures of populations in the future.

Crabbé et al. examined differential control of virulence genes in the yeast *Candida albicans* (athletes foot) in response to spaceflight (Crabbé, et al., 2013: PMID 24324620). They found that under spaceflight conditions virulence genes were activated, and that the yeast entered a hyphal stage that is indicative of an invasive state. Significantly, this study extended earlier observations with bacteria to another cell domain. Ongoing studies will elucidate the mechanisms and consequences of those changes. Microarray data are available at: https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-11/

In the past 5 years Space Biology has selected and funded 6 ground-based experiments that are in part designed to develop hypotheses to test in spaceflight experiments. A new study by Foster is the first Space-Biology-funded examination of a symbiosis involving a microbe and an animal in a reduced gravity situation (conducted in a rotating wall vessel). (Foster, et al., 2014: PMID 25370197). As these studies progress, Foster's group is helping to answer the question of whether microgravity will alter the animal host's innate immune response to beneficial microbes as it may with pathogens.

Since 2000,12 space flight experiments have been completed. For example, a jointly published NASA-Russia microbial survey of the Russian LADA vegetable production unit showed that the plants grown were microbiologically suitable for consumption by the ISS crew (Garland et al., 2010). In another study using hyper-gravity and spaceflight, Kimbrell's group showed that the effect of gravity is a continuum on the immune system and the virulence potential of opportunistic pathogens (Taylor, et al., 2014: PMID 24475130). This study exposed fruit flies to both bacterial and fungal pathogens and demonstrated impaired immune function in microgravity but enhanced-immune function under hypergravity conditions. See https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-1/ for microarray data.

Twelve ground-based experiments have been completed since 2000. At the foundation of the ability of organisms to detect gravity is the capacity to detect mechanical forces. To understand how the mechanosensitive channel of E. coli senses and responds to membrane tension is a question of critical importance. Paul Blount (Yang, et al., 2014: PMID 23416054) demonstrated that a single lipid interface residue controls the cell surface receptors that sense mechanical stimuli such as gravity.

Future Plans in the Space Biology Microbiology Element. An example of one direction for future efforts in this element is a recently selected and funded proposal by a team of PI's led by Cheryl Nickerson that will characterize the effects of long duration (~300 generations) spaceflight culture of isogenic strains of *Salmonella typhimurium* (wild type and Hfq-) on the genomic, epigenetic, transcriptomic, virulence and pathogenesis-related characteristics of these strains. The team will test the hypothesis that long-term multigenerational culture of *S. typhimurium* in the spaceflight environment will result in stress-induced heritable genetic and epigenetic alterations in *S. typhimurium*. The proposed work incorporates aspects of genetics, systems biology, and experimental evolution, and could advance our understanding of alterations in the genetics, epigenetics, and proteomics in *S. typhimurium* in response to long-term exposure to the space environment. While unique responses in microorganisms are well documented for short duration culture, the impact of long duration, multigenerational growth of microorganisms in the chronic stress of microgravity has not been investigated. The implications of this gap in knowledge are tremendous, as the response to short duration growth in microgravity alters gene expression, pathogenesis-related stress responses (e.g., biofilm formation and resistance to antibiotics) and virulence. Moreover, it is known that stress responses (including chronic stress) can increase mutagenesis in bacteria leading to genomic plasticity, which may provide novel mechanistic insight into genomic instability underlying resistance to antibiotics, pathogenicity and virulence.

Another currently funded Space Biology experiment is designed to understand the many challenges to mammalian physiological homeostasis present in the space environment that are known to create dysbiosis. This is important because immune or inflammatory changes, as well as metabolic alterations, observed with time in spaceflight may be attributable in part to dysbiosis. A team of PIs led by Fred Turek is using a rodent model to define the mechanisms by which time in space, diet, and host genotype interact to impact the composition of the gut microbiota; and how dysbiosis relates to gene expression and physiology in serum, colon, ileum, spleen, liver, and fat as well as the sleep/wake cycle and feeding behavior. This will be the first rodent microbiome study in space, and it will allow the investigators to measure gut inflammation, as well as various immune, and metabolic parameters. If all the aims are achieved, there will be substantial new information on the microbiota in space and how this correlates with gut permeability, immune function, and metabolic activities.

The effects of spaceflight on viral and microbial pathogen dynamics are largely unknown and represent both a significant gap in our knowledge and an important opportunity to study the presence of microbial populations and predict health risks during long-duration space exploration. So, a recently selected and jointly sponsored (by Space Biology and HRP) team of PI's lead by Crystal Jiang and David Bloom will conduct microbial censuses associated with various ISS modules using both traditional culture-based methods and state-of-the art molecular techniques. The objectives of the project are to measure presence of viral and select bacterial and fungal pathogens and correlate their presence on crew. This study will also specifically evaluate changes in the genomic and mutational diversity that is present in the Herpes virus virome present in crew. This study will address how the Herpes virus evolves in response to spaceflight conditions and will characterize the microbiome convergence of the crew and of the ISS. Both questions have significant ramifications for long duration spaceflight missions, and are a logical follow-on to the well-known results of Duane Pierson and colleagues on viral reactivation and shedding in astronauts during spaceflight.

Future Space Microbiology NRA's will solicit answers to the following critical questions to fill knowledge gaps in Spaceflight Microbiology, and to provide valuable data to GeneLab and to investigators developing translational tools:

- Can observations of altered virulence be confirmed through an infection model (on orbit) to result in increased mortality and morbidity (e.g. lower LD50)?
- Under the reduced microbial-diversity conditions of space habitats, do opportunistic pathogens have a greater survival capacity, and do they have a greater propensity to infect as compared with ground controls?
- Do symbioses such as N-fixation function in space as they do on earth? Do symbionts and commensals have the potential to become amensals or pathogens in space?
- How do communities of microorganisms (constructed or characterized assemblies) develop and persist over time? Are they stable? Does diversity always decrease? Do competitive interactions (2+ populations at a time) differ in spaceflight conditions from those on the ground?
- Does the microbiome of the crew converge during an increment? Is the rate of convergence different on orbit as compared with ground controls?
- Under liquid conditions do multi species biofilms also form unique biofilm structures such as observed with P. aeruginosa? Do such biofilms offer opportunistic pathogens a refugium in which they can grow to large numbers? Are antimicrobial substances able to penetrate biofilms on orbit similar to ground controls? How do biofilms in liquid culture influence the effectiveness of antibiotics and the development of antibiotic resistance in space? Do different antibiotic classes behave differently?
- Can astronaut dysbioses be remedied with probiotics?
- Can organisms be modified genetically to generate useful products or precursors and that will persist for long periods of time in space?

Technology Development for Space Microbiology experiments. A multi spectral fluorescence (RFP, GFP, YFP, etc.) microscopy capability is under development. It will have ports that can accept samples from the various microbial culture systems flown on the ISS, in order to be able to follow tagged microbes over time, and to examine microbial colonization over time. Ideally, this imager will be controlled by the PI on the ground.

Expected outcomes of Space Biology experiments in Microbiology include:

1. Increasing scientific knowledge and gains for space biology, commercial interests, and Earth-based academic and governmental life sciences communities will contribute omics data to GeneLab, which will lead to new ground-based and flight experiments. .

2. Demonstration of the space environment as an effective tool for facilitating translational research in coordination/ collaboration with HRP and other governmental agencies.

3. Data derived from the Space Biology microbiological science will be applicable to Earth-based biomedical questions and will lead to breakthroughs in understanding underlying mechanisms of diseases.

4. Data derived from the microbiology element will lead to new hypotheses and research opportunities that will foster the attraction of new academic- and commercial-based scientists to insure a positive future for space biology into future generations.

5. Data from the microbiology element will be applicable to Earth-based crop-production questions and will lead to breakthroughs in understanding underlying mechanisms to increase yield and nutritional content.

6. Space Microbiology studies will yield a better understanding of the frequency with which microbes become a greater threat to crew health through increased survival, virulence and drug resistance, and the mechanisms and triggers that incite those changes.

7. Space Microbiology studies will reveal how beneficial interactions among microbes and other organisms are affected by conditions in space, and how changes in those interactions affect important processes such as biodegradation or symbiotic nitrogen fixation with plants.

8. Space Biology efforts in microbiology will determine how complex communities of microbes develop and evolve in space in closed systems where opportunities to recruit new members or replace lost ones do not exist.

9. Studies within the microbiology element will show how simple and complex communities of microbes develop biofilm structures in space that have impacts on important processes related to health and to materials processing.

2) Cell and Molecular Biology Element

Goal. The overarching goal of the cell and molecular biology element is to determine how the space environment effects life at the cellular and molecular level. This element cuts across all science disciplines in Space Biology. Experiments will use state-of-the-art cell biology tools, and capitalize on the technological maturity, low cost, and speed of genomic analyses to monitor the evolution of genomic changes in microbes, plants, animals or other biological systems in response to the selective pressures present in the spaceflight environment. It derives its direction from Decadal Survey recommendations P2, AH2, AH3, AH5, AH14, AH16, CC8, and CC10.

Objectives included in this element are to use contemporary cell and molecular biology techniques and measures to determine and understand how cells respond and adapt to spaceflight changes (e.g., microgravity, radiation, stress):

a. Identify cellular and molecular mechanisms and associated factors and test observations and collect data across multiple cell types and specimen types to determine the universality or uniqueness of the conclusions

b. Characterize the effects of the space environment on DNA function and structure

c. Develop a systems biology-based understanding of the cellular and molecular changes to explain how gravitational changes in spaceflight effects organisms and causes phenotypic changes.

d. Characterize cell-cell communications, cell signaling, interactions with the environment (e.g. mass transport, efflux/uptake, etc) based on gravitational changes in spaceflight.

e. Determine how spaceflight affects the ability of eukaryotic cells to generate and maintain their complex internal cyto-architecture, including the cytoskeleton, and specialized membrane-

bound organelles and membrane domains that are integral to the regulation of both growth and form

f. Generate comprehensive genetic and omics data describing cellular molecular changes induced by spaceflight for deposit in the GeneLab database for open science access for the general science, commercial and public communities.

Guiding Questions and links to Decadal Survey Recommendations for Cell and Molecular Biology include:

- CMB-1 What are the underlying genetic, molecular and biochemical mechanisms of the cell that are influenced by gravitational changes and the space environment? (P2, AH3, AH5, AH14)
- CMB-2 What are the systems biology mechanisms and pathways for cell function that are responsive to gravitational changes and the spaceflight conditions? (AH3, AH5, AH8, AH10, AH14)
- CMB 3 What are the cellular and molecular bases for gravitational changes and space environment effects to tissue, organ, and whole organisms? (P2, AH3, AH4, AH5, AH8, AH15, AH16, CC8, CC10)
- CMB 4 How does the cell respond to the biophysical changes (thermal, fluids, etc) that occur due to microgravity and how does this impact overall tissue function and changes? (AH8, AH10, CC8)
- CMB 5 Does the space flight environment affect or influence cell and molecular functions causing tissue/organ dysfunction or disease states? (P2, AH2, AH3, AH8, AH10, AH14, CC8)
- CMB 6 Does the space environment affect cellular and molecular functions in a manner that impact tissue morphogenesis or development? (AH3, AH16, CC8, CC10)
- CMB 7 What are the driving space environmental factors that are sensed and responded to at the cellular and molecular levels? (P2, AH10, CC2, CC8)
- CMB 8 How does the space environment affect stem cell function and differentiation and how do the changes affect normal tissue function, regeneration, and embryogenesis (P2, AH3, AH8, AH14, CC10)

Space Cell and Molecular Biology History: For over 40 years, investigations have been conducted to understand how cell systems respond to the space flight environment. Early studies defined functional and morphology changes demonstrating multicellular organisms responded to the space flight environment at the cellular level (Sibonga, et al, 1989). The later development of sophisticated molecular biology analytical tools resulted in the identification of cell signaling pathways and molecular factors that specifically responded to changes in gravitational forces and the overall space flight environment. The combination of cellular and molecular biology has proven to be a cross cutting space biology element used to study biological processes from plants to animals (Cogoli, 2006; Paul, et al, PMID: 23281389). Some key areas of research are bone loss (LeBlanc, A.D., PMID: 17396004), immune dysfunction (Sonnenfeld, 2003; PMID: 12471311; Sonnenfeld, 2013. PMID: 23204258), and stem cell function and differentiation (Blaber, et al, 2014 PMID 25457968).

The induction of tissue loss due to mechanical unloading in microgravity has been hypothesized to be the result of stem cell dysfunction. To test this hypothesis, Blaber and colleagues investigated the effects of microgravity on mouse embryonic stem cell (mESC) function in the mouse embryoid body (EB) model system for studying tissue differentiation (Blaber, et al, 2015; PMID: 26414276). They found that 15 days of exposure to microgravity inhibited mESC differentiation and expression of terminal germ layer differentiation markers. Upon return to Earth, mESC cells recovered from space flown EBs were cultured in the lab, and the researchers discovered that these cells showed greater stemness. The findings from the study indicated supported the hypothesis and suggested a possible cellular basis for inhibition of tissue regeneration in space flight and for disuse conditions on Earth.

Studies to define the basis for altered immune function associated with the space flight environment have focused on investigating T-cell activation. Hughes-Fulford and colleagues have conducted extensive studies to uncover the underlying molecular mechanisms and factors responsible

for altered T-cell function in microgravity (Hughes-Fulford, et al, 2015; PMID 26276131; Martinez et al 2014; PMID: 25568077; Chang et al., 2012; PMID: 22750545; Boonyaratanakornkit et al., 2005; PMID 16210397). They discovered that the Rel/NF-κB pathway and transcription of immediate early genes required for T-cell activation were inhibited in microgravity. Follow-on space flight studies using the ESA Kubik hardware allowed for an onboard 1x*g* control. By comparing results between 1x*g*-, 0.5x*g*-, and microgravity-exposed T-cells, which were treated with or without mitogen for 1.5 hours, they discovered that expression of the mRNA translation regulatory microRNA, miR-21, was suppressed in the microgravity-exposed T-Cells; however, at 0.5x*g*, its expression was restored. Furthermore, they determined that a set of miR-21 mRNA target genes were similarly suppressed. These genes encoded factors required for T-cell activation. Based on the collective data, the team proposed a novel auto-feedback mechanism for suppression of normal early T-cell activation through build up of miR-21 to a critical level that results in inhibition of translation of key mRNA associated with T-cell activation.

Space Biology Cell and Molecular Biology Progress since 2010. Since 2010, there have been 9 flight and 8 ground-based Space Biology grants specifically to study cell and molecular biology in cell culture. However, the majority of other space biology flight and ground grants awarded during this time also had components of cell and molecular biology as a required component of their experiment objectives and analyses. Studies of specific cells and cellular/biochemical/molecular processes and integrative studies between cells and complex organisms have yielded important data on effects of spaceflight on biology and have led to the identification of candidate factors and processes that may be the sensors or regulators of the observed effects. The following are examples of these studies.

Understanding how microgravity contributes to inhibition of activation of the major immune cell, T-cell, is key to understanding immune dysfunction in the space environment. Dr. Hughes-Fulford and her group are seeking to identify the underlying biochemical and molecular mechanisms that contribute to T-cell activation. Her team determined the global gene expression profile differences between microgravity and 1xg that occurred in the early phases of T-cell activation, which led to the discovery that the Rel/NF-kB signaling pathway was the key pathway inhibited. Specific pathway analysis of genes that showed a two-fold or more reduction in expression revealed that TNF function may be the key gravity-dependent effector (Chang et al, 2012: PMID 22750545). The outcome from this study of this study led to a target underlying mechanism for defining immune dysfunction in the space environment.

Determining the dynamics and roles of various cellular organelles within cells is essential to understanding how the larger organism reacts and responds to microgravity. Dr. Rojas-Pierce and her group (http://dx.doi.org/10.4161/psb.29783 ; PubMed PMID: 25482812 , Dec-2014) are seeking to define the contribution of vacuolar and cytoskeletal dynamics to amyloplast sedimentation and gravitropic responses in shoots. Using an agravitropic mutant the team has recently reported that the impaired vacuole formation is the result of a mutation in a vacuolar trafficking protein resulting in multiple organelles instead of a large central vacuole. This protein has also been shown to regulate gravitropism and protein trafficking to the vacuole. Using a series of fluorescence microscopy techniques, demonstrated that the diffuse vacuoles are independent compartments and not connected to adjacent vacuoles, and that vacuole fusion is dependent on phosphoinosidides for vacuole fusion in plants (Zheng, et al., 2014: PMID 25482812).

Future Plans for Cell and Molecular Biology include an emphasis on expansion of space-based cell and molecular biology knowledge by developing a comprehensive understanding of the specific cellular and molecular factors and mechanisms affected by the space environment using systems biology to define changes to biological networks and to understand "re-wiring" networks within a cell and how these changes influence normal tissue and organ function, and whole organism well being. Studies in this element will:

- Develop a detailed and comprehensive picture of intra-cellular functions and biochemical and molecular biological processes affected by or that respond to spaceflight.

- Identify key effectors (biochemical, cellular, molecular) of cell sensing and response to gravity and the space environment.
- Develop a comprehensive cell-based network map of individual and integrated biochemical and molecular pathways showing how they are affected by gravity and the space environment.
- Use cell biology research to understand intra-cellular and inter-cellular communication and signaling to effect greater tissue and organ functions.
- understand cellular differentiation, tissue development, regeneration, and whole animal development in the space environment using in vitro-based stem cell research.
- Describe how changes in gravity loading cause biological dysfunction and deregulation in the space environment and Earth-based applications using cell and molecular biology research results in the GeneLab data-base.
- Utilize the data in the GeneLab data base for comparative studies with data from other specimens

Future Space Cellular and Molecular Biology NRA's will solicit answers to critical questions to fill knowledge gaps in Spaceflight Cell and Molecular Biology, and to provide valuable data to GeneLab and to investigators developing translational tools:

- How is the cell cycle and its regulatory factors affected by the space environment and what roles do these effects play on observed organism function and phenotype?
- What cellular factors, organelles, etc sense and respond to gravity and how do these activities change when organisms are exposed to the space environment?
- Does the space environment cause epigenetic changes, which genes are susceptible or affected, and how does this affect biological function in space and after return to Earth gravity?
- What are the long term cellular and molecular biology effects of long duration space environment exposure and what does this information reveal about overall organism adaption?
- What are the immediate early responses of a cell that occur when it transitions from a 1xg environment to microgravity?
- How does a cell respond to spaceflight radiation and do factors of the space flight environment influence the response?
- Are the space environment effects observed at the cell and molecular level specific to certain specimen populations or universal in nature?

Technology Development for Cell and Molecular Space Biology experiments. The technology gaps and requirements to enable space-based cellular and molecular biology research were identified by a panel of cell and tissue culture experts that NASA convened in June, 2013. The expert panel included NASA internal and external science community cell science experts. The recommendations by the panel were published, *Enabling Cell Science Requirements* (NASA/TP-2013-217379) document. Recommendations were made to provide the following key on-orbit capabilities in cell and molecular biology:

- Microscope system with illumination techniques, magnification, and fluorescence capabilities suitable for in situ imaging of cultures for monitoring health and analyses
- On-orbit habitat centrifuge for 1xg controls and partial g studies
- Rapid freeze capability for preserving proteins, transcriptional and metabolic products for ground omics analyses. This capability needs to mimic conditions on the ground to ensure high fidelity omics data.
- Slow freeze capability for stabilizing cells in stasis, this will enable investigators to pause an experiment on orbit and create an aliquote of cell and microbial cultures. Slow Freezing is conducted over the course of 12 hours and prevents the formation of ice crystals in the media. When done successfully, cell lines can be thawed and re-initiated both on orbit and on the ground.

- A research platform for conducting real-time quantitative gene expression analyses aboard the ISS. The Wetlab-2 system has been developed by Ames to better enable spaceflight genomic studies involving a wide variety of bio-specimen types. The instrument is planned for flight to the ISS in 2016.
- Culture chambers/hardware systems that provide stable and profusion environments capable of supporting cultures of different sizes and volumes and 3-D tissues; additional capability for smart chambers capable of directly monitoring/detecting cell functions and visualization (e.g. lensless imaging chips)
- Flow cytometry for cell quantitation, sorting, and analyses on-orbit
- Technology and techniques for increased and improved data collection, data dissemination, deeper analyses, and data access within the GeneLab data system
- Continuous GeneLab data system improvements so that the data base remains current with continued advancements in omics and bioinformatics techniques and presentation/analysis methods.

Expected outcomes of Space Biology experiments in Cell and Molecular Biology include:

1. Increased scientific knowledge gains for Space Biology, commercial interests, and Earth-based academic and governmental life sciences communities will contribute omics data to GeneLab, which will lead to new ground-based and flight experiments.
2. Expansion of systems biology knowledge for understanding and explaining how life is affected by gravity and the space environment at the cellular through whole organism levels
3. Use of spaceflight as an effective tool for translational biology for NASA HRP and commercial/private biomedical industry applications, such as biotechnology discovery biology, therapeutic discovery, therapeutics validation and tissue regeneration, and wound healing product development
4. Identification of underlying cellular and molecular mechanisms responding to and regulating the response of biological species to gravitational changes and the space environment that can be applied to reducing the medical risk to human exploration of space beyond low Earth orbit.
5. Data derived from Space Biology science will be translatable to Earth-based biomedical questions and lead to breakthroughs in understanding underlying mechanism to diseases,
6. The data derived from the cellular and molecular analyses will lead to new hypotheses and research opportunities that will foster the attraction of new academic- and commercial-based scientists to insure a positive future for space biology into future generations.

3) Plant Biology Element:

Goal. Understand plant and microbial growth in spaceflight environments and physiological responses to those environments by performing Space Biology experiments.

Objectives. Based upon the NRC Decadal Survey recommendations (P2, P3), this element will enable research to analyze plant growth and plant-microbe interactions in spaceflight and will investigate the physiological responses of plants to the multiple stimuli encountered. Understanding these responses will be invaluable for defining how multiple biological systems respond to spaceflight. It will also provide key information required for the eventual incorporation of plants and microbes into a bioregenerative life support system and will have critical impact on understanding effects of importance to human exploration of space, such as possible changes in virulence of plant and animal pathogens in space.

Guiding Questions and links to Decadal Survey Recommendations.

- PB-1 How does gravity affect plant growth, development & metabolism (e.g. photosynthesis, reproduction, lignin formation, plant defense mechanisms)? (P1, P2, P3)

- PB-2 Does the spaceflight environment cause alterations in plant-microbe interactions? (P1, P2, P3)
- PB-3 How can horticultural approaches for sustained production of edible crops in space be both improved and implemented (especially as related to water and nutrient provision in the root zone)? (P1, P2, P3)
- PB-4 What are the effects of chronic exposure to cosmic radiation on plants? (P1, P2, P3)
- PB-5 How do plants sense and react to gravity and what are the molecular mechanisms involved? (P1, P2, P3)
- PB-6 What implications are there from completing at least two complete plant life cycles in altered gravity environments relative to developmental patterns, structure, physiology, and reproduction? (P1, P2, P3)

Space Plant Biology History. *Over the past 50* years, researchers have pushed back many of the barriers to the successful growth of plants in space regarding lighting requirements, humidity and temperature control, and atmospheric considerations (the lack of convective mixing, elevated CO2 levels, ethylene and other volatile organic compounds present in cabin air). Researchers have confirmed that photosynthesis proceeds normally under microgravity conditions when adequate light, water, and nutrients are provided to the plants, and NASA-sponsored ground studies have shown that hydroponically grown crops can surpass world record yields, with commercial growers adopting some of these methodologies. Several plant species have been grown "seed-to-seed" in space (e.g., Arabidopsis, wheat, and soybean), and some plants seeds were returned to Earth and then sent back to space for second generation studies. (Mashinsky, et al., 1994: PMID 11540174; Levinskikh, et al., 2001: PMID 11668959; Link, et al., 2003: PMID 14686438). Watering systems for plants have been designed, and validated in space, but t is this area where major challenges still remain for the optimization of plant growth in microgravity since the lack of convective mixing has implications for the movement of water, oxygen and solutes through the root zone. As a consequence, most plants returned from microgravity have experienced some degree of hypoxia stress, and diffusion-limited movement of solutes have been shown to affect nutrient uptake by the roots.

The ability to incorporate the unique treatment of microgravity into experimental designs (not possible on Earth), has allowed researchers to discover new aspects of plant physiology and metabolism heretofore unknown (with implications for the enhancement of agriculture on Earth). NASA researchers have uncovered new aspects of the basic perception, transduction, and response of plants to gravity. Spaceflight experiments have validated Charles Darwin's theory of circumnutation (wobbling around the plumb line) in growing plant stems (Johnsson, et al., 2009: PMID 19320838). The thresholds to gravity for several plant species have been defined. The amounts and spectral quality of light for phototropism (bending toward light) have been tested in microgravity without the presence of the stronger, confounding force of gravity that is ever present on Earth (Millar, et al., 2010: PMID 20298479; Kiss, et al., 2012; PMID 22481136). By preserving plant tissue retrieved from spaceflight, space biologists have been able to detect differential expression of genes between the micro-g and 1-g environments and draw inferences on the types of enzymes and metabolic responses that occur in plants while growing in space (Paul, et al.. 2013: PMID 23919896). It has also been found that space-grown plants have shown an increased susceptibility to pathogen infection analogous to suppressed immune function in animals (possibly due to alterations in cell wall composition (Ryba-White, et al., 2001: PMID 11427686).

Space Plant Biology Progress Since the Previous Plan. Since 2010 there have been 14 NRA-selected grants for plant spaceflight experiments and 5 NRA-selected grants for plant ground studies. Recent results have revealed several profound discoveries, 3 of which follow.

(1) ***While plants don't have a brain or nervous system***, they can sense changes to their environment, such as lower oxygen levels caused by flooding, the munching of insects, or when they have been given a shake. NASA researcher Simon Gilroy and his team have discovered an "information highway" within the model plant Arabidopsis that can quickly send a signal from root to leaf tip through the movement of calcium ions. He and his colleagues engineered *Arabidopsis* plants so that cells

would glow green when calcium levels were low and red when they increased, enabling them to see a wave of calcium traveling through certain layers of the plant's cells all the way to its top. This was the first time scientists could see such a calcium signal moving through a plant, traveling at a speed of about eight cells, or a few millimeters, per second (Choi, et al., 2014: PMID 24706854). Their work has identified molecular targets that will let scientists tailor plants to grow better in space and on Earth.

(2) ***Since Darwin's observation in 1880 that plant roots wave and skew,*** it has been dogma that these movements were dependent on the interplay of gravity and touch responses as roots traversed a growth surface. However, in 2009 the first real-time images of roots growing along the surface of an agar plate on the ISS demonstrated that Darwin's major assumption about the role of gravity was wrong: gravity is clearly not required for waving and skewing. Images sent from the ISS showed that the Arabidopsis roots growing in the ABRS GFP Imaging System grew "down" the plate while skewing even more strongly than the comparable ground controls (Paul, et al., 2012: PMID 23217113).

(3) ***Phototropism experiments conducted in microgravity*** were able to reveal (using the centrifuges of the European Modular Cultivation System, on ISS) a red-light-based phototropism in Arabidopsis seedling stems that had previously been masked by the normal 1-g on Earth (Millar, et al., 2010: PMID 20298479). Also, reduced or fractional gravity studies showed an attenuation of the red-light induced phototropism at gravitational accelerations ranging from 0.1 to 0.3 g (Kiss, et al., 2012; PMID 22481136). These studies showed that some flowering plants may have retained a red-light sensory system for phototropism, a discovery that has implications for understanding the evolution of land plants since directional red-light responses can be can be seen only in 1-g within older plant lineages such as mosses and ferns.

Future studies already selected for flight will investigate topics such as:

(1) Epigenetic Change in Arabidopsis thaliana in Response to Spaceflight Differential Cytosine DNA Methylation of Plants on the ISS. Anna-Lisa Paul, University of Florida. Epigenetic features play a role in regulation of gene expression and subsequent response of an organism to its environment. DNA methylation is a major epigenetic modification that is not directly coded into the genome sequence, and yet can modify expression and be inherited for at least one generation. This experiment will use a genome-wide approach to define changes in DNA methylation that occur in Arabidopsis during spaceflight. Elucidating spaceflight plant methylomes contributes to understanding how biology responds and adapts to spaceflight, and also provides insight into adaptive strategies plants use in the face of novel stimuli outside of their evolutionary experience. This will be the first plant spaceflight experiment to directly investigate how the plant methylome contributes to space adaptation.

(2) Spaceflight-Induced Hypoxic/ROS Signaling. Simon Gilroy, University of Wisconsin – Madison. This experiment will be an integrated transcriptomics approach using Arabidopsis. It will address complex questions related to plant growth, signaling, and hypoxic responses both in spaceflight and on Earth in addition to testing whether the hypoxic Ca^{2+} signaling system provides targets for genetically engineering potential countermeasures to low oxygen stress, which will have direct implications for growing plants in space and on the ground.

(3) Using Brachypodium distachyon to Investigate Monocot Plant Adaptation to Spaceflight. Patrick Masson, University of Wisconsin-Madison. Major cereal grain crops are monocots (one of two classes of Higher Plants), yet most investigations of plant adaptation to the spaceflight environment have been carried out on the dicotyledonous model plant *Arabidopsis thaliana*. It is unknown whether conclusions from such studies can be extrapolated to monocotyledonous plants. To fill this knowledge gap, these experiments will investigate growth, development and transcriptome profiles of *Brachypodium distachyo*n seedlings under spaceflight conditions, and compare these adaptive responses to those documented for *Arabidopsis* seedlings. Lines chosen have altered gravitropic responses.

(4) Characterizing Plant Gravity Perception Systems. Scot Wolverton, Ohio Wesleyan University.
There are two gravity sensing mechanisms in higher plants: (1) the primary statolith based system, and (2) a non-statolith based sensory system. The aim of this spaceflight experiment is to understand how the two gravity sensory systems, and their molecular components, interact by using plants strains with and without statoliths. The expected results will be important elements in future understanding of gravity perception in higher plants. This flight experiment can be expected to profoundly impact our understanding of the mechanisms of plant gravity sensing.

(5) An integrated Omics Guided Approach to Lignification and Gravitational Responses: The Final Frontier. Norman Lewis (Washington State University, Pullman).

This project uses transcriptomics, proteomics, metabolomics and phenomics and will be extremely important to the GeneLab Initiative, since it is a "Space Biology design reference mission". The aim is to better understand the crosstalk between lignification carbon metabolism and gravity sensing and signaling. The overall goal is to evaluate whether decreased lignin would improve carbon concentrating mechanisms and/or water use efficiency in plants grown in space. The relevance for space flight is that plants (or crops) that do not put a lot of energy into making lignin could have higher productivity because they could allocate those carbon resources into plant growth. This comprehensive omics approach is expected to provide basic insights into fundamental mechanisms in biological systems and generate a data resource that can be utilized by other scientists.

Technology Development for the Plant Biology Element. It is a goal of the NASA Space Biology Program to extend the capabilities of the currently available spaceflight hardware to perform cutting edge plant research in space. There are four plant-related technology development projects underway.

1) Advanced Plant Habitat (APH). Objective**:** Develop a large volume plant habitat for multi-generational studies in which environmental variables (e.g., Temperature, Relative Humidity, Carbon Dioxide Level, Light Intensity and Spectral Quality) can be tracked and controlled in support of whole plant physiological research and Bioregenerative Life Support System investigations.

2) Biological Research in Canisters-Light Emitting Diodes (BRIC-LEDs). Objective: Extend the capability of the BRIC Petri Dish Fixation Unit (PDFU) hardware to support ISS-based investigations on plant, and other, autotrophic organisms requiring light plus in-flight fixation with a minimum of crew time.

3) Multispectral Fluorescent Imager (MFI). Objective: Develop a hardware capability to perform automated real time fluorescence imaging (requiring no crew time) for use in the microgravity environment for plants and other biological specimens. There is a requirement that this be contained within a reliable and controllable environment for science investigations.

4) SporeSat: Objective: Develop a NanoSat with the capability of flying fern spores (or alternative model systems) and measuring the development and changes associated with calcium currents during germination and development in different gravity environments. The hardware that has been developed can be flown on free-flying expendable "cube-sats", or adapted for use in experiments on ISS or other vehicles where return is possible.

Future Plans and Research Solicitations for the Plant Biology Element include:

1) Determine how plants adapt to spaceflight from the molecular to whole plant level using existing hardware (e.g. EMCS, Veggie, BRICs) on ISS.

2) Provide better understanding of molecular and genetic levels of plant adaptation to spaceflight by bringing on-line and conducting yearly (at a minimum) investigations within the Advanced Plant Habitat and using the enhanced research capabilities (BRIC-LED, Multispectral Fluorescent Imager) to expand the scope of plant topic areas available for research.

3) Answer basic research questions about plant biology that will enable the future development of a bioregenerative life support system for use by humans in space exploration.

4) Continue efforts at improving microgravity watering systems for plants (the primary challenge for growing plants in space).

5) Augment and expand ISS capabilities, reduce risk, and decrease demands on ISS crew time by developing research campaigns that use a wide variety of research platforms appropriate for Space Biology science investigations. .

a) Micro-g Simulators – Ground based micro & partial g simulation devices to negate directional influence of gravity (e.g. 2D & 3D Clinostats, Micro-g Bioreactor, Random Motion Machines).

b) Antarctic Balloon Flight Campaigns for deep space radiation investigations.

c) Parabolic Flight Campaigns - short duration (20-30 s) micro-g and partial "g" investigations.

d) Suborbital Flights (2-5 minute micro-g investigations).

e) Free flyer campaigns requiring no crew interaction (e.g. CubeSats).

f) Secondary payloads on expendable rockets to explore responses to deep space.

Expected Outcomes. Some expected outcomes of studying plants in space are as follows:

1. **Relevance to Earth-Based Agriculture:** Data derived from Space Biology plant science will be applicable to Earth-based agriculture by leading to breakthroughs in understanding underlying mechanisms related to basic signaling pathways that are in place to ensure stress survival in hostile environments, increased production rates, and nutritional content. The outcome will be improved management of natural resources on Earth, and implementation of sustainable agriculture in marginal lands.
2. **Insights Gained on Aspects of Plant Physiology:** Insights gained through studying plants grown in microgravity will trigger breakthroughs in related areas relevant to the growth of plants on Earth. For example: (1) There will be an improved understanding of the role of gravity in determining fundamental characteristics of plant growth and development. (2) We will gain a better understanding of the interaction between light and gravity in regulating plant growth. (3) Results will address the fundamental question of the role of mechanical loading on plant growth and development, and probe how genes might be used to tailor plant growth to thrive both in the unique environment of space and on Earth.
3. **Insights Gained on the Regulation of Plant Gene Expression:** The transcriptomic, proteomic and epigenomic data generated by plant spaceflight experiments will be used to construct models of plant metabolic pathways responsive to spaceflight. Knowledge gained will be translated into corresponding gene functional studies (e.g. mutant analysis and overexpression), which will lead to the discovery of new genes that modulate plant growth and development both in microgravity and on Earth.
4. **Development of Bioregenerative Life Support Systems:** Plant spaceflight experiments will help to further define the impacts of spaceflight on biological systems to better enable NASA's future space exploration goals. New knowledge of how to grow plants for incorporation into Bioregenerative Life Support systems will enable development of plant strains better adapted to producing food, oxygen and clean water for long-duration space missions. This knowledge will be beneficial for the design of large plant habitats for planetary surface missions.
5. **Insights Gained on Plant Cellular Processes:** Plant spaceflight experiments will advance knowledge on fundamental questions of (1) how gravity modulates the internal cytoarchitecture & molecular processes that govern the development of plant cell walls, (2) how plant cells perceive, process and transmit mechanical stimulation, and (3) other cellular processes, ultimately producing basic knowledge to guide genetic engineering efforts to generate plants that are better adapted to the weightless environment of space and increased production on Earth.
6. **Relevance to Crew Nutrition for Long Duration Missions:** NASA's Human Research Program (HRP) understands that there is great value in adding fresh foods on a regular basis to the crew's diet. In partnership with HRP, Space Biology is developing protocols for growing vegetables in space, and making assessments of the nutritional quality of those vegetables grown.

7. **Relevance to Crew Psychology for Long Duration Missions:** There is a known potential for providing a positive effect on the crew's well-being by having plants in their environment. The Veggie hardware has started to provide the ISS crew with the sights and smells associated with growing their own vegetables, and this activity will expand in the coming years.
8. **Insights Gained On Plant Microbe Interactions:** Through both basic and applied plant research on ISS, assessments of plant-microbe interactions & microbial food safety issues are being addressed, and will continue to add to our knowledge of these key areas.
9. **Insights Gained on Hypoxia Responses in Plants:** Plant spaceflight experiments have directly advanced our understanding of hypoxic responses in plants grown in both space and on Earth in addition to testing whether the hypoxic Ca^{2+} signaling system provides targets for genetically engineering potential countermeasures to low oxygen stress, with potential for counteracting the detrimental effects of flooding on Earth.
10. **Insights Gained on Strain Development for Optimizing Plant Performance:** Findings from plant spaceflight experiments will continue to contribute to the understanding of basic signaling pathways that are in place to ensure stress survival in foreign environments, thus making possible the design and growth of plants that are resistant to stress in space and on Earth.
11. **Relevance of Plant Results to other Multicellular Organisms:** Because of the conservation of stress sensing and response mechanisms across multicellular organisms, results from plant spaceflight experiments will have implications for the general understanding of stress and in the design of solutions for space stress management in all multicellular organisms, including humans.
12. **Inspiration for Research and Outreach on Earth:** Results derived from the plant element will lead to new hypotheses and research opportunities that will foster the attraction of new academic and commercial-based scientists as well as the upcoming generation of students to insure a positive future for space biology into future generations.

4) Animal Biology Element

Animals, both vertebrate and invertebrate are frequently used to model human disease. The most commonly used model organisms for which genomics are now well defined include vertebrate species, e.g., rodents, both rats and mice, and a variety of invertebrate species, e.g., nematodes and insects. NASA has used model organisms extensively to evaluate human spaceflight hazards, elucidate the fundamental mechanisms life uses to adapt to microgravity, and apply such knowledge to advance human exploration, and for societal benefits on Earth. The Animal Biology Element provides the scope, recent research progress, and future plans for the animal research component of this plan. The following goals, objectives, and guiding questions apply to both vertebrate and invertebrate animal groups; however, the remaining sections of this element are divided between vertebrate and invertebrate groups for clarity, i.e., to distinguish the research progress, current activities and future plans for each animal group.

Goal. The goals of the Animal Biology element are to understand the basic mechanisms animals use to adapt to the spaceflight environment, to alterations in gravity in general, and to contribute basic knowledge of biological adaptation to spaceflight in order to accelerate solutions to biomedical problems affecting human exploration of space as well as human health on Earth.

Objectives. The objectives of this element are to:

- Determine whether artificial gravity provides a multi-system countermeasure to the deleterious effects of prolonged spaceflight, e.g., musculoskeletal atrophy, cardio-pulmonary deconditioning, immune system dysfunction including abnormal fracture repair and wound healing.
- Determine gravity "thresholds" for various important physiological functions using a centrifuge to produce "G" levels between micro- and Earth-gravity, which can only be done in spaceflight.

- Enhance communication, cooperation, and collaboration with NASA's Physical Sciences and Human Research programs to accelerate Space Biology discoveries and sharing them with the NASA and extramural communities.
- Elucidate the animal organ, tissue, and system responses to gravity changes and the genomic and regulatory mechanisms driving them.
- Determine how the space environment affects animal physiology, e.g., immune function and wound healing, metabolism, sensory-motor control, behavior, organ structure and function, and aging.
- Apply Systems Biology approaches to elucidate the combined effects of radiation and microgravity on animal survival, physiology, reproduction, lifespan and aging.

Guiding Questions and links to Decadal Survey Recommendations for this element are:

- AN-1 How does the space environment affect basic physiological functions and the applicable regulatory mechanisms and pathways, e.g., sensory-motor control, behavior, vision, metabolism, organ structure and function, aging and lifespan? (DS: CC7, AH2,3,5,8.13.14.15) Are there sex differences in the physiological responses? (CC10)
- AN-2 Do microgravity and ground-based microgravity analogs alter radiation damage and repair mechanisms in animals? (DS: CC8, 9)
- AN-3 How do animals sense and respond to alternations in gravity, i.e., what cellular and molecular mechanisms and pathways are used? (DS: CC2, AH9, 10)
- AN-4 Is there a "g" threshold (force and duration) that can mitigate the biological effects of microgravity? (DS: CC2, AH9, 10)

4a) Animal Biology - Vertebrate

History of Space Biology Research on Vertebrates. Other than pocket mice that were sent to lunar orbit on Apollo 17 in 1972, NASA conducted no studies of spaceflight effects on rodents until US/Soviet joint experiments on the Soviet Bion biosatellite missions beginning with Bion 3 in 1975. In 1977, on the Bion 4 mission, US/Soviet collaborative experiments with rodents included the use of centrifugation to provide a 1g acceleration to the animals throughout the 18.5d flight. Post-flight analyses of the animals provided evidence showing a protective effect of artificial gravity on the microgravity-induced weakening of the femur and also promoted a faster post-flight recovery of bone structure and strength losses. However, the relatively short radius and small cage size potentially mitigated the overall beneficial effects of artificial gravity during the flight. (Spengler DM, Morey ER, Carter DR, Turner RT, Baylink DJ. Effects of spaceflight on structural and material strength of growing bone. Proc Soc Exp Biol Med 1983; 174:224-228.)

Group-housed rats (*Rattus norvegicus*) were first flown by NASA in a US built Animal Enclosure Module (AEM) placed in Shuttle middeck lockers on STS-8 in 1983. Individually-housed rats were flown in a Research Animal Holding Facility on the STS (51b) Shuttle/SpaceLab (SL-3) mission in 1985. Improvements were steadily made in the AEM over its use on 27 STS missions providing the inspiration for the development of the Rodent Research Habitat launched for 30 days to the ISS on SpaceX-4 in 2014. A complementary 30 day flight with US/Russian collaborative rodent experiments occurred on the Bion M1 mission in 2013. The two 30 day flights provided opportunities to compare the effects of spaceflight on female mice (ISS) and male mice (Bion-M1) although the different habitat environments on the Bion and ISS flights provided less than the ideal comparative opportunity.

There have been many articles written on the specific effects of spaceflight on animals. The following are a few overview articles of the history of animal research in space: of the effects of spaceflight on animals can be found in the following articles: 1) Holton, E., E. Hill, and K.A. Souza. Animals in Spaceflight: From Survival to Understanding. J Musculoskeletal Neuronal Interactions 2007; 7(1) 17-25, 2) Dubbs, C and Burgess, C. (2007) Animals In Space: From Research Rockets to the Space Shut-

tle. Springer. ISBN 0387360530, and 3) NRC report, 2011: Recapturing a Future for Space Exploration, Animal and Human Biology, pp. 99-203, http://www.nap.edu/catalog/13048.html.

Progress Since the 2010 Space Biology Science Plan. The focus of Space Biology Animal Research over the past 5-10 years has been on the musculoskeletal, cardiovascular and immune systems. Over the past decade NASA selected 42 experiment proposals using vertebrate animals. Two of that total flew on the Russian Foton M3 mission in 2007 using newts (*Pleurodeles walti*) or geckos (*Pachydactylus turneri*), but the remainder of the experiments used or proposed to use rodents, either rats or mice. As is the case with ground-based biomedical research, rodents are often the vertebrate animal of choice for space studies. The following are examples representative of the broad multi-disciplinary nature of the rodent experiments flown and the results obtained:

A recently published study by E. Almeida/E. Blaber using mice flown on the Space Shuttle found that in addition to well-known increased bone resorption by osteoclasts there is evidence for two other pathways leading to bone loss. One involves destruction of bone tissue by osteocyte cells that are a structural element of bone (osteocytic osteolysis). The other pathway involves disruption of the normal bone-forming cell cycle. The study also found that the pelvic and femoral regions of the mouse skeleton are active sites of microgravity-induced bone loss. These results suggest that defining methods for restoring normal cell signaling pathways to encourage bone production for astronauts could be a promising area of translational research in the future (Blaber, et al., 2013: PMID 23637819) (DS: AH2,3)

Published results from pioneering ground-based studies in mice, by M. Bouxsein and others, has created a pathway to an ISS flight study based on their validation of a significant bone loss countermeasure drug's efficacy. The protein Sclerostin is known to be involved in blocking bone formation during unloading conditions. An experimental antibody to Sclerostin (SCIABII) was injected in mice during simulated microgravity (hindlimb unloading) for 21 days and compared to control animals. Amazingly, the Sclerostin antibody was so effective in preventing bone loss in the simulated microgravity animals they appeared to have healthier bone tissue than the controls that experienced no unloading. If confirmed, in a flight experiment flown on the Space Shuttle STS-135 flight, and proven safe for human use, such a drug has the potential to help age-related bone loss including osteoporosis and will provide benefits for long duration human spaceflight (Spatz, et al., 2013: PMID 23109229). (DS: AH4)

An intriguing study was conducted by M. Mednieks and recently published with the goal to develop a simple saliva-based assay that could be used in both mice and humans to identify biomarkers for the stresses associated with spaceflight. Mouse and human salivary glands are similar in morphology and physiology and allow regular non-invasive biosampling. The mouse subjects enabled detailed study of the secretory proteins that may respond to the spaceflight environment. The study was conducted on three Shuttle flights (STS-131,133, and 135) with female mice and crew sampling; a similar study using male mice was flown on Bion M1. The findings indicate that the salivary proteome associated with beta-adrenergic hormone regulated responses is significantly altered during spaceflight. Importantly, a number of unrelated proteins showed no change. These data will support design of a biochemical test system to identify specific salivary proteins that can track stress levels in animals and humans with inevitable applications to Earth biomedicine, as well (Mednieks, et al., 2014: PMID 24984624). (DS: CC10)

Strong interest exists to understand better cerebral blood flow and vascular resistance in spaceflight, especially based on the evidence accumulating that human vision is degraded by long-duration spaceflight that may be associated with these factors. Human headward fluid shifts in microgravity are well documented but study of the responsiveness of the vascular system is not well understood. M. Delp led a study of the basilar arteries of mice on three Shuttle missions and the 30-day Bion M1 mission using in vitro functional, mechanical and histological structural analysis. The

physical attributes of the arteries were not different between experimental groups but there was clear microgravity-related attenuation of both vasoconstrictor and vasodilator properties that could limit the range of vascular control of cerebral perfusion and impair the distribution of brain blood flow during periods of stress (Sofronova, et al., 2015: PMID 25593287). This result clearly needs to be further studied in animals with the goal to translate the results to humans and clarify their potential impact on related chronic vision problems associated with spaceflight. (DS: AH8,9)

Immunosuppression is seen in healthy astronauts who have flown in space; however little is known about the mechanisms that cause the reduced immunity in spaceflight. An experiment conducted by M. Hughes-Fulford onboard the STS-131 mission was designed to elucidate the role of T-lymphocytes in mounting a response to an antigen during spaceflight. Female mice were implanted prior to flight with osmotic pumps that delivered antigen early in the 15 day mission launched in April, 2010. The results suggest that the early T-cell immune response was inhibited in animals that received antigen early in the flight. Even 24 hours after return to Earth the immune response still reflected a delayed T-cell response. Gene arrays were conducted and confirmed that T-cell activation was altered by spaceflight and since T-cells act as the "quarterback" of the immune response, they appear to play a key role in the immuno-suppression seen in animals and astronauts during spaceflight (Martinez et al., 2015: PMID 25568077) . (DS: AH13,15)

Future Plans for Vertebrate Animal Studies. Following the NRC Decadal Survey recommendations, NASA recently selected 17 experiments using mice for flight on the ISS in the near future. For the most part, these new experiments emphasize the musculoskeletal and vascular systems, and are designed to build an integrated picture of how vertebrate physiology changes in space, and how physiological changes are inter-related. Experiments are selected to provide basic Space Biology science results that have potential translational impacts to human biology. A few of the 17 that are currently in the definition phase of their development for flight are profiled below along with their anticipated results.

A. Robling, "Foundational in Vivo Experiments on Osteocyte Biology in Space": Bone loss in astronauts due to microgravity is an increasing health concern as the mission durations increase. Mice will be studied on the ISS for short (5 weeks) and long (16 weeks) durations. Bone osteocyte-related changes in DNA, RNA, protein, using a Systems Biology "omics" approach will be measured and compared to ground based space flight analog models to clearly define similarities and differences between what is found in flight and control conditions. Tissue-level changes in bone mass, turnover dynamics and material properties will be measured to compare genotypic and phenotypic adaptations. The experiment should confirm the utility of ground-based analogs for simulating spaceflight microgravity effects on bone thus increasing the value of ground-based research options. It is anticipated that new knowledge will be gained about microgravity effects on osteocyte biology and its role in mechano-sensing. (DS: AH2)

R. Globus, "Free Radical Theory of Aging in Space": The similarities between the weeks to months-long adverse physiological effects of spaceflight on the crew, and the decades-long process of aging on Earth, intrigues many gerontologists. The potential link at the molecular level between lack of effective gravity and aging effects has been proposed for study using transgenic mice. The mice will be engineered to have less oxidative damage in their mitochondria (associated with fewer free radicals) that may occur in both long-duration spaceflight and progressive aging. Both wild-type and transgenic mice will be flown or kept as ground controls for up to two months. Then, neural, cardiac, bone tissues will be recovered for analysis of gene expression, oxidative stress biomarkers and tissue function. Preliminary ground studies suggest that the differences between the two mouse types will likely be significant and interpretable. The study should identify basic mechanisms behind molecular degenerative changes in both spaceflight and normal aging, providing a potential application both to NASA exploration and terrestrial biomedicine. (DS AH8 and CC10)

E. Almeida, "The Role of p21/CDKN1a in Microgravity-induced Bone Tissue Regenerative Arrest: A Spaceflight Study of Transgenic p21/CDKN1a Null Mice in Microgravity": Previous work by this investigator, with his colleague E. Blaber, has identified the p21 gene as a likely mediator of bone regeneration arrest in microgravity (Blaber, et al., 2013: PMID 23637819). This study will determine the extent of regenerative cell proliferation in adult stem cells in the presence (Wild Type mice) and absence (Transgenic mice) of the p21 gene. Tissues will be studied known to be impacted by spaceflight including the pelvis, femur and tibia using various approaches from gene array analysis to micro-computed tomography for structure analysis. This study done in vivo with whole organisms will complement a parallel study by E. Blaber using the new NASA BIOS cell culture system on the ISS. Both will focus on the role of the p21 gene but one focused more on whole organism bone and the other on cell culture stem cells from bone marrow to study differentiation and mineralized bone formation. This informal team approach should produce a powerful set of combined results. (DS: AH3) (DS: AH3)

S. Chapes, "Collection of Immune/Stress-Related Tissues from Mice Flown on the ISS." The B cell antibody response to a tetanus antigen immunization will be studied during spaceflight as a key measure of the immune system status in mice. Key tissues related to B cell responses will be collected from frozen samples postflight including; liver, bone marrow, thymus and the adrenal glands. This suite of tissues taken from mice immunized during flight will provide new knowledge about the relationship between immunization, stress and the immune response in microgravity. The translational potential to human crew from such an integrated study is deemed to be high. (DS AH14, AH15)

R. Robbins, "Vascular Health in Space": This study will focus on microRNA (non-coding RNA with role in gene regulation) and its role in vascular repair during spaceflight. Mice will undergo preflight surgery that produces an abdominal aortic injury (non health-related) and all animals will be returned after 30 days for postflight analysis. The goal is to study a) the microRNA response in injured versus normal vasculature, b) the efficiency of the vascular repair including smooth muscles and c) the adequacy of the associated angiogenesis. This study provides unique results associated with the need to better understand wound healing in general and future needs to address medical emergencies and associated recoveries in the astronaut crew. (DS: AH8)

Future Solicitations for Animal Research: Vertebrates. Future plans include:

- the use of artificial gravity, i.e., centrifuges on the ISS or other space platforms as a potential Earth-gravity countermeasure to biomedical problems associated with spaceflight as well as a tool to study "fractional" gravity levels, between micro- and Earth gravity, to answer basic questions in gravitational physiology, e.g., What are the gravity thresholds that will trigger a response in a variety of model organisms? And how do living systems respond to lunar and mars gravity levels? Such studies are not possible on Earth, only in spaceflight.
- parallel experiments will be solicited using ground-based centrifuge facilities at Ames Research Center to augment the flight experiments and provide ground-based hyper-g data in the "Gravity as a Continuum" project, as well as to develop new hypotheses for test in spaceflight.
- the use of Systems Biology approaches, gathering multi-omics data under well-documented conditions to determine how the omics changes are related to one another, and how they produce physiological and behavioral adaptive strategies. Omics data will be made public in the "open science" Genelab data base so that investigators can develop new hypotheses to be tested in spaceflight to accelerate and amplify discoveries for both human exploration and societal benefits
- the identification of gender differences in the way vertebrate animals respond to the spaceflght environment.

New Technologies for Animal Research: Vertebrate. A Rodent Research Facility and associated support equipment have been developed for the ISS that accommodates mice and with plans to include rats in the future. It was flown to the ISS on SpaceX-4 and the basic technology and related procedures were validated. Support systems include Rodent Transporter for flights to the ISS,

an Animal Access Unit to move animals to either the Rodent Habitat or the General Purpose Workstation for animal handling/processing. On-orbit centrifuges for rodent research on the ISS are in development by JAXA (Mouse Habitat Unit) and NASA via a NASA Small Business Innovative Research contract (SBIR). These systems when developed and validated will enable high-priority fractional gravity and inflight 1-g controls to be done with rodents.

An on-orbit rapid freeze capability is needed to preserve vertebrate tissue structure and molecules of genomic, proteomic, and metabolomics analyses. New advances in biotelemetry are also needed for more in depth probing of animal physiology and also for better monitoring of animal health and well being.

4b) Animal Biology – Invertebrate

Recent History of Research with Invertebrates. Over the past decade there have been 7 flight experiments conducted with invertebrates; one host-pathogen study with Drosophila melanogaster-*Escherichia coli* or *Beauveria bassiana*, flown on the Space Shuttle, one neurosensory/behavior study with snails flown jointly with Russian co-investigators on the Foton M2 and M3 missions, and one host-pathogen on orbit infection study with *Caenorhabditis elegans-Salmonella typhimurium*, flown on the ISS. The remaining 4 experiments are in flight definition phase. In addition, there were 4 ground-based studies selected and completed. They used spaceflight analogs to examine s stress responses and mechanoreception in *C. elegans* and *D. melanogaster*.

Progress since the 2010 Space Biology Science Plan: Examples. The results of a Drosophila infection experiment flown for 12 days on the Space Shuttle demonstrated that spaceflight impacts the ability of an invertebrate host to fight a parasitic infection. The ability of fly larvae to phagocytize *E. coli* in culture was attenuated following spaceflight, and in parallel, the expression of genes involved in cell maturation of Drosophila immune surveillance cells (Hemocytes) was down-regulated. In addition, the level of expression in larvae was significantly reduced in genes involved in pattern recognition receptors that specifically recognize bacteria, and immune stress genes, hallmarks of innate immunity. In adults, the efficiency of bacterial clearance measured *in vivo* following infection with *E. coli* post-flight, remained robust. In summary, spaceflight altered immune responses in *Drosophila* and that disruption occurs in multiple interacting pathways. (Marcu, et al., 2011: PMID 21264297)(DS: AH14).

Extending the aforementioned Drosophila post-flight infection study to in-flight infection, C. Nickerson flew cultures of *C. elegans* to which were added small quantities of *Salmonella typhimurium* thus providing, for the first time, an opportunity to study host-pathogen infection during spaceflight. The analysis of this exciting experiment is still in progress. (Flown Dec. 2014) This experiment is expected to add significantly to our knowledge of the combined action of cellular, molecular and biochemical networks in the host that potentiate transition to disease in response to infection. This is a critical issue as spaceflight negatively impacts crew immune function and alters microbial virulence, gene expression and antibiotic resistance. (DS: AH 14,15, P2

A noteworthy collaborative US/Russian experiment was flown for approximately two weeks aboard the unmanned Russian biosatellites Foton 2 and 3 by R. Boyle (NASA) and his Russian co-investigators. These experiments were a continuation of Boyle's studies to determine how the linear acceleration-sensing vestibular (balance) organs adapt to spaceflight. Using the terrestrial gastropod snail *Helix lucorum* major findings included:

- the latency of body position change to sudden orientation change was significantly reduced in the snails when tested following recovery.
- significant differences in HPeP gene mRNA expression pattern in statocyst receptor neurons were observed postflight between flight and ground-control snails.
- a significantly larger firing rate response in statocyst neurons to body position changes at all tested speeds were observed in flight snails during post-flight testing.

- responses of the statocyst receptors to adequate motion stimulation in the postflight snails were independent of the motion direction while in the control animals some differences in responses to different directions were observed

These results suggest that the use of this model organism can provide an opportunity to describe the subcellular mechanisms of changes in gravi-receptors due to microgravity exposure. (Balaban, et al., 2011: PMID 21479267)

Future Plans for Invertebrate Animal Experiments: Using the NRC Decadal Survey as a guide, several experiments with invertebrate model organisms were selected recently after peer review and are planned for flights on the ISS over the next few years. The experiments are in flight definition and are described below including their expected outcomes:

- **R. Bodmer: "The effects of microgravity on cardiac function structure and gene expression using the Drosophila model"** - *D. melanogaster* cultures will be flown on the ISS for multiple generations and preserved at different stages of development. Detailed analyses of the development of the heart will be conducted postflight.(DS: AH8, AH 16). Little is known about how spaceflight affects early embryonic development and in particular heart development. Since congenital heart defects are the most frequent birth defect in the United States, and because future planned space missions include extended duration flights with the long-term prospect of colonization of the moon or mars, it will be important to know how microgravity impacts establishment of the primary body axes and subsequent cardiac development in the embryo.

- **S. Govind: "Does spaceflight alter the virulence of a natural parasite of Drosophila".** Spaceflight is known to affect host-pathogen interactions and in some instances shown to make pathogens more virulent. This could make humans more susceptible to infection during long-duration spaceflight. Previous studies of immune function in fruit flies have led to critical advances in the understanding of immunity in humans as well as other animals. This experiment will study the combined effects of altered pathogenesis of a natural parasite of fruit flies and the corresponding response of an infected host in the space environment. This mission will be launched with a combination of samples consisting of flies alone and samples containing flies and parasitic wasps (*Leptopilina*). At the conclusion of the mission, all samples will be returned alive and utilized in a variety of experiments designed to investigate how the immune system of the fruit fly hosts responded to the wasp parasites, as well as how the virulence of the wasp parasites changed due to spaceflight. Host defense and microbial virulence will be measured by using a set of well-established assays in the fruit fly and wasp models. These experiments will help uncover the underlying mechanisms that regulate the balance of the host-pathogen interaction and determine whether this balance is shifted due to spaceflight.

- **T. Boothby: "Using water bears to identify biological countermeasures to stress during multigenerational spaceflight"** - The tardigrade, *Hypsibius dujardini,* will be cultured on the ISS and sustained over multiple generations. Samples will be taken periodically during the flight and preserved for extensive global gene expression analyses postflight. This unique organism can tolerate extraordinary stresses, including the temperature extremes, vacuum, and radiation of space. In addition, it can survive extensive periods in a state of "suspended animation". This experiment will identify both immediate and multigenerational changes in global gene expression induced by spaceflight and should provide insights to how the organisms resist extreme stress. Such insights may provide novel applications for human exploration as well as to biomedical problems on Earth. I(DS: AH16)

- **S. Vanapalli: "Determining muscle strength in space-flown *Caenorhabditis elegans*".** The small nematode worm, *C. elegans,* is a good model for studying the effects of spaceflight on muscle. The proposed experiment will fly cultures of *C. elegans* on the ISS and utilize a novel technology to measure changes in muscle strength over time in space. The technology employs a Velcro-like mat with fibers that measure the force the worms exert as they deflect the fibers while crawling through the mat. *(***Note:** A complementary muscle experiment with C. elegans was selected by

ESA, T. Etheridge, U. Exeter. NASA and ESA will coordinate the development and flight of the two experiments.) Systems Biology "omics" methods will be coupled with the muscle strength sensor data to make *C. elegans* a much more complete and comprehensive genomic model for muscle physiology thereby enabling meaningful translation of discoveries in *C. elegans* to astronauts and to the population at large. (DS: AH5)

Future Solicitations for Animal Research: Invertebrates. Using guidance provided by the NRC Decadal Survey, high priority studies with invertebrate animals will be solicited in future Space Biology NRAs including Systems Biology studies to:

- investigate the effects of spaceflight on radiation damage repair following a preflight sub-lethal exposure to radiation. (DS: CC8)
- determine the mechanisms and time course of adaptation to alterations in gravity, from micro-g and fractional-g through hyper-g (DS: CC2)
- elucidate the effects of spaceflight on reproduction, development, and aging across multiple generations of invertebrate model organisms

New Technologies developed or under consideration to Support Animal Research include:
A centrifuge is under development for use on ISS that will support and augment research with insects and other small organisms. The centrifuge will provide opportunities to investigate how invertebrates sense, respond and adapt to fractional gravity levels, e.g., moon and Mars levels.

b. A centrifuge for rodent research aboard the ISS is under consideration. As with the small organism centrifuge mentioned above, a centrifuge for rodent research will provide an opportunity to determine how complex organisms respond to accelerations along the gravity continuum, i.e., from microgravity to hypergravity and also identify potential gravity thresholds (force and duration) that can provide clues as to how artificial gravity might be used for a systems level countermeasure to the deconditioning associated with spaceflight in animals and humans.

c. NASA ARC is reactivating its ground-based Center for Gravitational Biology Research facilities that include a variety of centrifuges and other acceleration devices, to support a "Gravity as a Continuum" science campaign. This activity will include ground-based and flight research to determine the response of life to gravitational force across the entire g-spectrum, micro- to hyper-gravity.

d. Fruit Fly Habitats: A variety of systems are being developed to provide flight opportunities for research with insects, initially, *D. melanogaster* to determine how spaceflight affects the immune system, radiation damage and repair, reproduction, development and aging.

Expected Outcomes of Space Biology Experiments in Animal Biology (vertebrate and invertebrate) include:

1. New knowledge from pioneering studies of animals at fractional gravity levels provided by centrifuges flown on the ISS and other platforms as well as ground-based will stimulate new insights into the mechanisms of how life senses, responds, and adapts to gravity levels different from any experienced during their evolution on Earth. Results will contribute to the potential use of artificial gravity as a biomedical countermeasure for long duration human spaceflight.

2. Expanded uses of the data for scientific, commercial, and medical applications resulting from the large increases in animal "omics" data submitted to "open science" databases, e.g., GeneLab.

3. Identification of gender differences in the response of animals to spaceflight will provide new insights into developing gender-specific approaches to biomedical countermeasures for long duration spaceflight as well as for ground-based applications to human health and performance.

4. Significant advances in the understanding of the cellular and molecular mechanisms of animal physiology, e.g., musculoskeletal, immune, cardiovascular, sensory-motor systems, etc, will translate to applications for human health issues in space and on the ground, e.g. neuromuscular degenerative wasting diseases and biomedical problems associated with aging.

5. Animal experiments in Space Biology will contribute significantly to the education and training of the next generation of scientists, engineers, and space explorers.

5) Developmental, Reproductive and Evolutionary Biology Element

Goal. Determine how the space environment affects the processes of development, reproduction and evolution of life. (The Decadal Survey recommended that "Studies should be conducted on transmission across generations of structural and functional changes induced by exposure to space during development. Such research will provide vital fundamental information about how genetic and epigenetic factors interact with the environment to shape gravity-dependent processes and about the penetrating influence of these factors across subsequent generations. Spaceflight experiments offer unique insights into the role of forces omnipresent on Earth (but absent in orbital flight) that can actively shape genomes in ways that are heritable. Such spaceflight experiments would place gravitational biology at the leading edge of modern developmental and evolutionary science. Ground-based studies should be conducted to develop specialized habitats to support reproducing and developing rodents in space. This research could be accomplished within 10 years." (AH16)

Objectives of this element include:

a. Elucidate the mechanisms of development, from gametes to mature adult, that are influenced by altered gravity environments and other factors in the space environment

b. Determine how gravity and the space environment influence processes of reproduction in complex organisms

c. Understand how long-term exposure, over many generations, to altered gravity and other factors in space can lead to evolutionary changes in species

Guiding Questions and links to the Decadal Survey for this element include:

- DEV-1 What are the mechanisms by-which the space environment influences how individual eukaryotic cells carry out genetically defined programs of proliferation, differentiation and development into specialized cells, tissues and multi-cellular organisms? (P2, AH3, AH8, AH14, CC10)
- DEV-2 What are the systems biology mechanisms, pathways and molecular networks, through all developmental phases of life, that are altered by factors of the space environment? (P2, AH16, CC10)
- DEV-3 Do organisms that are raised in microgravity develop normally, *i.e.*, structurally, physiologically, behaviorally? (P2, AH16, CC10)
- DEV-4 Is gravity required for normal development of gravity sensing organs in plants and animals and/or for reproduction, development and maturation of organisms throughout their lives? If so, how much gravity, for how long, and, for what periods of the life span, is gravity required? (P2, AH16, CC2, CC10)
- DEV-5 How does the space environment influence reproductive health in both male and female organisms and what are the differences between the sexes? (P2, CC2, CC8 CC10)
- DEV-6 What are the mechanisms of change in reproduction, lifespan and the aging processes for organisms that live their lives in the space environment? (P2, AH16, CC2, CC8 CC10)
- DEV-7 Can complex organisms, plants and animals, be grown successfully through many generations in altered gravity environments? If not, why not? (P2, AH16)
- DEV-8 Are changes in development and reproduction, expressed in space, persistent over multiple generations after return to Earth, or are they reversible? (P2, AH16, CC2, CC8 CC10)
- DEV-9 How are the processes of natural selection and evolution influenced by the space environment over long periods of time and over many generations? (P2, AH16)

Recent History of Research in this element includes:

Reproduction, development and evolution encompass a broad spectrum of biological studies and investigations that start with the genesis of reproductive cells, progressing through all stages of cellular division and differentiation from gametophyte to adult and, finally, addressing the genetic and epigenetic changes, handed down over many generations. Over 40 successful spaceflight and ground-based research investigations in areas of developmental and reproductive space biology, for both plant and animal species, were conducted during the 1990's and early 2000's. A notable example was the first successful fertilization and development in space of a vertebrate species. Female frogs onboard the Space Shuttle were induced to ovulate, sperm was added, and the eggs fertilized normally, demonstrating that gravity-induced rotation of the fertilized zygote was not necessary to develop a normal body plan and a free swimming tadpole (Souza, et al., 1995, PMID: 7892210). Also notable was an experiment onboard the MIR space station that demonstrated seed-seed reproduction and development could occur during spaceflight and that multiple generations of plants in microgravity are possible (Musgrave et al, 1998). Gravitational and Space Biology Bulletin 12: 56). Among the most prominent recent findings from those investigations include the discovery of a gravity detection-related gene in Drosophila that also plays an important role in development (Bjorum, et al., 2013; PMID 23620738). Several developmental biology studies were also conducted on rodents, revealing changes in sensorimotor functioning and thyroid development (Walton, et al., 2005: PMID 15774538; Adams, et al., 2000: PMID 10710385). Several important contributions related to developmental biology of plants were also made during this period including embryonic development and seed development (Kuang, et al., 2005:,PMID 15747444; Popova, et al., 2009: PMID 19938622). More recently, funded research has focused on the processes of cellular and tissue differentiation. A recent mini-review of stem cell health and tissue regeneration in microgravity describes recent findings, as well as important contributions made prior to the year 2000 (Blaber et al., 2014: PMID 25457968).

Progress since 2010 in this element: examples. Since 2010, there have been 22 funded investigations that have a significant component of study in a field related to the Developmental, Reproductive, and Evolutionary Biology science element for Space Biology. Several current investigations seek to understand the mechanisms of differentiation and proliferation of stem cells exposed to the space environment. These investigations include studies of tissue-regenerative matrix kinase (E. Almeida), osteogenic differentiation of somatic stem cells (E. Blaber) and differentiation processes of neural stem cells (P. Espinosa). Current investigations involving reproductive biology include studies of both male and female reproductive health in mice (J. Tash) and an investigation of immune system impacts on reproductive function and mammary development in mice (J. Pluth). Current plant and invertebrate model organism research studies also explore the underpinnings of tissue differentiation in adult organisms. Plant studies are using next generation sequencing technologies to look at mechanisms of seed development (I. Perrera) and mechanism of differentiation of undifferentiated cells (R. Ferl). Research on invertebrates includes a C. elegans study of muscle differentiation (S. Vanapalli). Several multi-generational investigations are also beginning to follow the changes in invertebrate species including C. elegans (C. Sabanayagam), water bears (T. Boothby) and fruit flies (R. Bodmer) as a result of long-term exposures to the space environment. Finally, there are even a few newly-awarded investigations that will follow bacterial species over a number of generations that can begin to elucidate the evolutionary adaptation of species when cultivated under the unique selective pressures of space (C. Everroad, C. Nickerson). The NASA Task Book provides more information on these and other related past and currently-funded investigations in this science element (https://taskbook.nasaprs.com/Publication/welcome.cfm).

Future Plans in this element include:

The most critical questions to address in the coming years span a wide array of developmental, reproductive and evolutionary processes. Over the coming decade multigenerational studies of higher organisms (both vertebrates and invertebrates) will serve as a critical driver for many of the

developmental and reproductive biology research investigations. Studies to determine the mechanisms of differentiation for cells, tissues, plants and animals over a single life span, or many generations will be needed. Gender differences, aging and life span changes are also of critical importance to study over the next decade. New technologies need to be developed for multigenerational studies of invertebrate and vertebrate organisms and long term evolutionary studies of microbial life. One of the highest priority recommendations of the NRC Decadal Survey calls for studies to be conducted on transmission across generations of structural and functional changes induced by exposure to space during development (AH16).

Future Solicitations for Research in this Element include as a high priority:

a Study model organisms across their respective reproductive and developmental phases using artificial gravity, i.e., centrifuges on the ISS or other space platforms as a potential countermeasure to biomedical problems associated with spaceflight and also as a tool to answer basic questions in developmental gravitational physiology, e.g., How does gravity shape processes of reproduction and development across the lifespan and generations? What are the gravity thresholds that trigger reproductive and developmental responses in key model organisms? Can living systems develop and reproduce at lunar and mars gravity levels?
b. Use Systems Biology approaches, including the GeneLab Project open science methodologies, to accelerate and amplify discoveries in developmental and reproductive science that are relevant to human exploration and promote Earth benefits
c. Identify sex differences in the way that developing and reproducing animals respond to the spaceflight environment?

<u>New Technologies required to support Developmental, Reproductive and Evolutionary Biology research</u>:

a Animal habitats are required that support rodents in all phases of reproduction and development. It is likely that multiple habitats with different capabilities will be needed to accommodate the requirements for multi-generations in space, i.e., breeding, birthing and nursing, maturation and aging.
b An Advanced Plant Habitat that will accommodate multiple generations of small plants is required with appropriate sensors and recording capabilities to monitor plant heath during the full plant growth and reproductive cycle. (See Plant Biology Element)
c. Habitats that support multi-generations of invertebrate animal species are required to support gravitational biology research i.e., reproductive, developmental and evolutionary biology studies. (See Animal Biology Element, section 4b)
d. Space-based centrifuges are needed to provide a 1 g experimental control environment and also that can provide fractional-g accelerations to determine if reproduction and development can proceed normally in the absence of 1g and at fractional g levels, e.g., levels approximating those on the moon and Mars.
e. NASA ARC has been authorized to reactivate its ground-based Center for Gravitational Biology Research facilities to support a future "Gravity as a Continuum" science campaign that will include ground-based and flight research on reproducing and developing organisms.

<u>Expected Outcomes of Space Biology Experiments in Developmental, Reproductive and Evolutionary research</u>.

a) New insights into the mechanisms of how organisms reproduce and develop in response to gravity levels different from any experienced during their evolution on Earth provided by pioneering studies of reproducing and developing animals at fractional gravity levels provided by centrifuges flown on the ISS and other platforms will stimulate.

b) Exponential increases in understanding resulting from large increases in "omics" data on reproducing and developing organisms submitted to "open science" databases, e.g., GeneLab, will contribute to expanded uses of the data for scientific, commercial, and medical applications.
c) Identification of sex differences in the response of animals to spaceflight will provide new insights into developing sex and/or gender-specific approaches to biomedical countermeasures for long duration spaceflight as well as for ground-based applications to human health and performance.
d) New knowledge of the effect of spaceflight on reproduction and development resulting from completing multigenerational studies with one or more model organisms, e.g., simple invertebrate animals, e.g., nematodes and fruit flies, small plants, e.g., *Arabidopsis thaliana, Brassica rapa,* and small vertebrates, e.g., rodents.
e) Establishing an "omics" database of key stages in reproduction, development and evolution that occur during altered gravity conditions in several model organisms, e.g., nematodes, fruit flies, rodents, and plants
f) Significant advances in the understanding of the cellular and molecular mechanisms underlying development of physiological systems, e.g., reproductive, musculoskeletal, immune, cardiovascular, sensory-motor systems, etc, and inform human health issues in space and on the ground, e.g. reproductive and developmental disorders, including degenerative wasting diseases, and aging.
g) Experiments on animal reproduction and development in space will contribute significantly to the education and training of the next generation of scientists, engineers, and space explorers.

D. Vision and Goals of Space Biology 2016-2025

Space Biology Vision. NASA's Space Biology will expand the scientific knowledge base for both space application and for the improvement of life on Earth. The new knowledge will contribute to NASA's ability to predict biological health and reduce engineering risks during long-term space exploration. Space Biology research results will have a critical impact on understanding the biological effects important to human exploration of space.

Space Biology Goals for 2016-2025

1) Create new knowledge of how biological systems adapt to space that can translate into benefits for NASA Exploration Missions and for Earth applications through sponsoring competitively solicited Space Biology research.;
2) Leverage and amplify ISS and ground-based Space Biology research findings for Space Life Sciences researchers through the use of state-of-the art omics and other molecular/systems biology tools to populate a GeneLab data base.
3) Maximize cutting-edge Space Biology research that answers the NRC Decadal Survey's recommendations by building a diversified research portfolio using ISS, free-flyer, sub-orbital, ground-based analogues and centrifuges, or other appropriate venues.;
4) Advance knowledge for societal benefit and contribute to human exploration of space by steadily building a larger, more robust, and internationally competitive United States Space Biology scientific community;
5) Develop cutting edge technologies to facilitate conduct of biological research in spaceflight;
6) Accelerate new knowledge/results by a) engaging and building a robust U.S. Space Biology community, and b) soliciting research using regularly occurring NRA's that will answer the NRC Decadal Survey's recommendations of NASA in Space Biology;
7) Work with international partners and other U.S. agencies to achieve NASA's objectives; and
8) Train and inspire a new generation of U.S. Space Biologists.

Achieving the goals. Space Biology will enable NASA to achieve the goals of fundamental and translational biology research in space that is critical to the Agency's exploration and space commercialization missions. Research will be selected to provide the best results, at the lowest cost, in the shortest time. Space Biology research will include ground-based investigations, microgravity investigations on parabolic and suborbital platforms, and free flyer and ISS flights. Spaceflight experiments will include both primary and secondary payloads, such as cubesats or missions involving biospecimen sharing, as required. The development of centrifugation capabilities on Earth and in space, to identify specific responses both on the ground and in flight will be a hardware focus during the next few years. To address the critical science questions for exploration and long term manned spaceflight and to synthesize the appropriate conclusions from complex integrative life science research, multidisciplinary research teams will need to be formed as necessary.

To study gravity as a continuum from micro-gravity to hyper-gravity requires space-based centrifuges for both basic research and astronaut countermeasures. To optimize the scientific use of space, and to prepare humans for exploration, this requirement has been discussed for decades. Recommendations for such centrifuges have come from NASA's advisory committees, as well as the national and international space life sciences communities (National Research Council reports, 1979, 1987, and 2011). Space-based centrifuge facilities provide the only practical way to apply controlled accelerations for significant durations to organisms, including humans. Using a centrifuge one can vary the force, duration, and frequency of loading to find the optimal combination to produce a countermeasure prescription for maintaining human health and performance during spaceflight. Space-based centrifuges can also provide a 1-g control condition for organisms that experience the same rigors of launch and re-entry stress as an experimental group of organisms exposed to microgravity during spaceflight. In addition, Space-based centrifuges are uniquely suited for research to

evaluate biological responses to fractional g levels, including the 1/6-g and 3/8-g of the moon and Mars, respectively. Only with space-based centrifuges can questions of g-thresholds and responses between 0 and 1g be addressed. For example, Dr. John Kiss, U. Mississippi, has used the European Modular Cultivation System (EMCS) onboard the ISS to determine how germinating small plants (*Arabidopsis thaliana)* respond to different levels of gravity and also to different levels and frequencies of light. He has recently identified a novel cellular and molecular mechanism never seen in higher plants. He showed that the roots of *Arabidopsis* respond (bend toward) red light, a phenomena that had only been previously observed in primitive plants like moss and ferns. This new discovery in fundamental plant physiology, was made possible through the use of the EMCS' variable g centrifuges and the unique microgravity environment onboard the ISS. Dr. Kiss' discovery is providing important information for developing bioregenerative life support systems with plants for potential long-term space missions. Dr. Kiss' experiments included reduced, fractional gravity, studies that showed an attenuation of the red-light induced phototropism at gravitational accelerations ranging from 0.1 to 0.3 g (Kiss, et al. 2012: PMID 22481136). Those studies showed that during evolution some flowering plants may have retained a red-light sensory system for phototropism, a discovery that has implications for understanding the evolution of land plants since directional red-light responses can only be seen in 1-g within older, more primitive plant lineages. Another novel Space Biology plant experiment is looking at g-thresholds and the molecular mechanisms of fern spore germination during spaceflight. Dr. Stan Roux, U. Texas, and colleagues is using a small automated centrifuge system to measure changes in calcium currents in spores germinated during spaceflight while rotating on a centrifuge. Results from this experiment will shed light on the molecular mechanisms that drive plant germination and the g-thresholds that stimulate it.

Unfortunately, a space-based centrifuge for vertebrate animal research has been flown only once. Rodents (male rats) were flown in 1977 on the 18.5 day Soviet Cosmos 936 mission that included two centrifuges each containing 5 rodent habitats with a single animal per habitat. The centrifuges were mounted in the ~3 m diameter spacecraft that also included 10 animal habitats mounted in a fixed location within the spacecraft thus exposing the animals to microgravity during flight. The animal habitats on the centrifuges were mounted at a radius of 32 cm and spun at 53.5 rpm to provide a 1-g acceleration that was applied during the duration of the spaceflight. Analyses of the centrifuged and non-centrifuged animals shortly post-flight showed that centrifugation prevented the loss of bone strength in the long bones. Surprisingly, reduced bone growth occurred in both groups; however, the centrifuged animals showed a much greater bone growth rate post-flight indicating a more rapid recovery than occurred in the animals exposed to microgravity. Space-based centrifuges that will accommodate rodents are currently being developed by both the Japanese Space Agency, JAXA and by NASA with the JAXA system expected to fly on the ISS with a payload of mice within the next year.

Space Biology Products. The scientific, technology and human capital products and accomplishments of Space Biology will span a wide range of scientific, technical and public media outlets. Distribution of the products of Space Biology research will continue to be a focus and responsibility of the Space Biology Program. The GeneLab database will be a major repository of data on the biological response to the space environment. (http://genelab.nasa.gov/data/) Other sources of investigation data will include the task book (https://taskbook.nasaprs.com/Publication/) and the Life Science Data Archive (https://lsda.jsc.nasa.gov). The Space Biology team along with its funded investigators, will regularly bring results forward to the science and engineering community through an array of methods including publications, presentations and personal contact. The accomplishments from Space Biology will also reach the broader public media through press releases, news stories and other outlets when the results are newsworthy.

Data Management Plans. All current and future NRA's will include language that requires a data management plan in compliance with NASA and other federal regulations. Current PI's are working closely with the centers and the GeneLab team to identify synergies, and facilitate that deposition of data into the GeneLab Database as well as other databases.

Contributions to Exploration Products. Space Biology research results will contribute to Exploration goals, including technologies and results related to areas such as human health countermeasures, bioregenerative life support and commercialization of Low Earth Orbit. Since Spaceflight is conducted in a remote and isolated environment, understanding the ecological performance and evolution of the biologically active areas of space systems is critical to mission success. As knowledge is gained in the individual research areas the implications of those discoveries will be integrated into an overall system picture using the tools of systems biology. The balanced and stable performance of the ecological system is key to system performance and risk reduction in the operational Exploration systems and will drive significant society and commercial improvements. The exporting of significant finding to either the NASA community, the external research community, and/or the external commercial community will be a major program focus.

Increasing partnerships to facilitate productivity. Space Biology will continue to develop its connections with a network of NASA and international partners. Key research partners are NASA's Human Research Program and the Physical Sciences Program within SLPS within HEOMD, the Advanced Exploration Systems Program, Astrobiology within SMD, and Synthetic Biology and technology maturation efforts within STMD. NASA Space Biology Research will generate data needed by the external community, and the ISS National Lab. This latter community will include other federal agencies (NIH, NSF, USDA, DOD, DOE, etc). Our international partners will continue to be a two-way conduit of scientific finding and important collaborative work for mutual benefit. It is also expected that the Space Biology Program together with commercial entities and private foundations will be working together to achieve mutual goals.

Developing the next generation of Space Biologists. Development of the next generation of biological investigators will be a critical product of the Space Biology Program. This will include research fellowships for undergraduate, graduate and postdoctoral level professionals. Additionally, Space Biology will issue more New Investigation/ New Investigator awards and Biospecimen Sharing Opportunities to enable young investigators to enter into the pipeline. In our two recent NRA 10% of the Proposals were headed by either current postdocs or very junior faculty. Space Biology will also conduct a number of activities for students (K-12 and undergraduates) to professionals at a variety of levels through continuing education and other career development opportunities.

Challenges to Achieving the Goals. The degree to which the goals of NASA's Space Biology can be achieved over the 2016-2025 decade depend heavily on: 1) the development of a suitable cadre of ground-based investigators who conduct experiments that define hypotheses requiring spaceflight; 2) sufficient opportunities for spaceflight for experiments that have been deemed scientifically meritorious; 3) adequate funding to support experiment development, flight operations, and the necessary equipment to meet science objectives.

In order to accomplish its scientific goals, Space Biology must: 1) Maintain close alignment of NASA Space Biology objectives and research project goals with 2010 Decadal Survey priority recommendations; 2) comply with President Obama's Executive order "Making Open and Machine Readable the New Default for Government Information", enabled through the NASA GeneLab Project; 3) Develop more collaboration with other NASA programs, other federal agencies, CASIS, or international partners; 4) promote the use of in-flight centrifugation across all elements and species to determine gravity thresholds, responses to fractional Earth gravity such as will be experienced on Mars or moon, and to provide 1g controls in flight; 5) increase flight opportunities and the resources on ISS needed to maximize science return (Ability to accomplish goals & recommendations of NRC Decadal Survey is diminished by reduced access to ISS & reduced crew time availability for implementing NASA science); 6) Promote ground-based research and alternative spaceflight platforms. 7) Adopt a Systems Biology Approach in 50-70% of all Space Biology tasks and actively solicit users of the open science GeneLab database to develop new knowledge from spaceflight omics data: and 8) enlarge NASA's research community by outreach to major scientific professional societies

(e.g., Experimental Biology, American Society for Microbiology, American Plant Biology Society, American Society for Cell Biology.)

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