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GENERAL INFORMATION

Gravitational and Space Biology (ISSN 1089-988X) is a journal devoted to research in gravitational and space biology. It is published by the American Society for Gravitational and Space Biology, a non-profit organization whose members share a common goal of furthering the understanding of the biological effects of gravity and the use of the unique environment of spaceflight for biological research. *Gravitational and Space Biology* is overseen by a steering committee consisting of the Publications Committee, the Editor, the President, and the Secretary-Treasurer of the ASGSB.

The American Society for Gravitational and Space Biology was created in 1984 to provide an avenue for scientists interested in gravitational and space biology to share information and join together to speak with a united voice in support of this field of science. The biological effects of gravity have been acknowledged since Galileo's time, but only since the 1970s has gravitational biology begun to attract attention. With the birth of the space age, the opportunity for experimentation over the full spectrum of gravity finally became a reality, and a new environment and research tool became available to probe biological phenomena and expand scientific knowledge. Space and spaceflight introduced new questions about space radiation and the physiological and psychological effects of the artificial environment of spacecraft.

The objectives of ASGSB are:

- To promote research, education, training, and development in the areas of gravitational and space biology and to apply the knowledge gained to a better understanding of the effect of gravity and space environmental factors on the flora and fauna of Earth.
- To disseminate information on gravitational and space biology research and the application of this research to the solution of terrestrial and space biological problems.
- To provide a forum for communication among professionals in academia, government, business, and other segments of society involved in gravitational and space biological research and application.
- To promote the study of concepts and the implementation of programs that can achieve these ends and further the advancement and welfare of humankind.

MEMBERSHIP: The American Society for Gravitational and Space Biology welcomes individual, organizational, and corporate members in all of the basic and applied fields of the space and gravitational life sciences. Members are active in the fields of space medicine, plant and animal gravitational physiology, cell and developmental biology, biophysics, and space hardware and life support system development. Membership is open to nationals of all countries. Members must have education or research or applied experience in areas related to the Society's purposes: i.e., Doctorate, Masters with 2 years experience, Bachelors with 4 years experience (student members must be actively enrolled in an academic curriculum leading toward a career related to the Society's purposes), or special appointment by the Board of Directors. Membership applications may be obtained by writing the American Society for Gravitational and Space Biology, P.O. Box 2581, Chapel Hill, NC 27515, or at the society website (<http://www.asgsb.org>).

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American Society for Gravitational and Space Biology



Program and Abstracts for the **Twenty-fifth Annual Meeting**

November 5-9, 2009

Raleigh, NC

The ASGSB gratefully acknowledges the generous contributions offered to conduct this meeting by: the NIH/NIAMS and NCI, Grant 1R13AR057685-01, NASA, Grant NNX09AQ56G; and the following corporate sponsors: Science and Technology Corporation, Techshot, Lockheed Martin, Mains Associates, Bionetics, USRA and Orbitec.

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Short Program



SHORT PROGRAM – 2009 ANNUAL MEETING

Thursday, November 5 (Pre-Meeting)

12:00 noon – 6:00 PM	Registration Desk Open
7:00 PM	ASGSB Governing Board meeting

Friday, November 6

7:30 AM – 5:00 PM	Registration Desk Open
8:00 AM - 8:30 AM	Welcome and opening remarks
8:30 AM - 12:00 noon	Symposium I: Closed-Loop Regenerative Life Support for Sustainable Habitation in Space and on Earth <i>Chair: Cary Mitchell, Purdue University</i>
8:30 AM	Christophe Lasseur, European Space Agency
9:20 AM	Yasuhiro Tako, Institute for Environmental Sciences, Japan
10:05 AM	<i>Break</i>
10:20 AM	Kurt Preston, Army Research Office, Research Triangle Park, NC
11:05 AM	Ray Wheeler, NASA Kennedy Space Center
12:00 noon – 1:00 PM	Lunch, ASGSB committee meetings
1:00 PM – 2:00 PM	Town Hall Meeting: NRC Decadal Survey of the Biological and Physical Sciences in Space
2:00 PM – 5:00 PM	Poster Session I (Student poster competition)
5:00 PM – 6:00 PM	Committee Meetings
6:00 PM – 9:00 PM	Reception

SHORT PROGRAM – 2009 ANNUAL MEETING

Saturday, November 7

8:00 AM – 5:00 PM	Registration Open
8:30 AM - 12:00 noon	Symposium II: Biological Engineering <i>Chair: Marshall Porterfield, Purdue University</i>
8:35 AM	Wendy Boss, North Carolina State University
9:20 AM	Kristala L. Jones Prather, MIT
<i>10:05 AM</i>	<i>Break</i>
10:20 AM	Anthony Guiseppi-Elie, Clemson University
11:05 AM	Greg Copenhaver, University of North Carolina
12:00 noon – 1:00 PM	Lunch
1:00 PM – 2:00 PM	Special Lecture: "What is Required to Maintain Skeletal Muscle Health in Space?" Danny Riley, Medical College of Wisconsin
2:00 PM – 4:15 PM	Concurrent Oral Sessions I. Gravitational and Space Biology: Plants Chris Brown, <i>Chair</i> II. Biological Technologies and New Capabilities for Research and Spaceflight, Adarsh Deepak, <i>Chair</i>
4:15 PM – 5:30 PM	Poster Session II
6:00 PM – 9:00 PM	Banquet and ASGSB Business Meeting Keynote Speaker: Mary Schweitzer, North Carolina State University, "Dinosaurs and Space"

SHORT PROGRAM – 2009 ANNUAL MEETING

Sunday, November 8

7:00 AM – 8:30 AM	ASGSB Governing Board Meeting
8:00 AM – 12:00 noon	Registration Desk Open
8:30 AM – 12:30 PM	Symposium III: The ISS as a National Lab <i>Symposium Chair, Ken Souza, Logyx, LLC</i>
8:35 AM	Mark Uhran, NASA Headquarters
9:15 AM	Julie Robinson, NASA Johnson Space Center
9:55 AM	<i>Break</i>
10:10 AM	Cheryl Nickerson, Arizona State University
10:50 AM	Tim Hammond, Durham VA Medical Center
11:30 AM	Panel Discussion Moderator: Neal Pellis, Johnson Space Center
12:30 – 1:30 PM	Lunch
1:30 – 4:30 PM	Oral Session III: Space Biology and Physiology: Animals, Cells, and Microbes <i>Chair: David Tomko, NASA Headquarters</i>

Long Program



LONG PROGRAM – 2009 ANNUAL MEETING

Thursday, November 5

- 12:00 Registration Begins
- 19:00 ASGSB Governing Board meeting

Friday Morning, November 6

- 7:30 Registration Opens
- 8:00 Welcome and opening remarks

Scientific Symposium I
Closed-Loop Regenerative Life Support for Sustainable Habitation in Space and
on Earth

8:30 - 12:00

Cary Mitchell, Symposium Chair

Start		Page
8:35	MELiSSA: The European Project of a Closed Life Support System. C. Lasseur, J.D. Brunet, H. De Weever, M. Dixon, C.G. Dussap, F. Godia, M. Mergeay, D. Van Der Straeten, W. Verstraete. [1]	2
9:20	CEEF: Closed Ecology Experiment Facilities. Y. Tako, R. Arai, S. Tsuga, O. Komatsubara, T. Masuda, S. Nozoe, K. Nitta. [2]	2
10:05	<i>Break</i>	
10:20	Sustainable Base Camp System: Required Capability for the Army Future Force, 2030 & Beyond. K. Preston. [3]	2
11:05	The Past, Present, and Future of Bioregenerative Life Support for Space Habitation. R.M. Wheeler. [4]	2
12:00	Lunch, ASGSB Committee Meetings	
13:00	Town Hall Meeting: NRC Decadal Survey of the Biological and Physical Sciences in Space	

LONG PROGRAM – 2009 ANNUAL MEETING

Friday Afternoon, November 6

Poster Session I: Student Poster Competition

14:00 – 17:00

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I-1	A Highly Sensitive Nanocube Augmented Carbon Nanotube-based Lab-on-a-chip Platform for Continuous Astronaut Health Monitoring. J.C. Claussen, A. Ul Haque, T.S. Fisher and D.M. Porterfield. [5]	4
I-2	Noninvasive Measurement of Root Auxin Flux using a Self-referencing IAA Microsensor. A. Diggs, E. McLamore, P.C. Marzal, J. Shi, J. Claussen, A. Murphy and M. Porterfield. [6]	4
I-3	The Role of <i>DREB2B</i> in Gravitropism and Phototropism. C. Johnson, P. Kumar and J.Z. Kiss. [7]	4
I-4	Strategies for Cloning of the <i>GRAVITY PERSISTENCE SIGNAL (GPS) Genes.</i> B. Justus, N.M. George, C. Schenck, C. Bruggeman, D.R. Luesse and S.E. Wyatt. [8]	4
I-5	Transplantation of Bone Marrow Stromal Cells Cultured under Simulated Microgravity into a Spinal Cord Injury Rat. M. Takeda, T. Magaki, A. Sasaki, T. Manabe, M. Matsumoto, Y. Kawahara, L. Yuge and K. Kurisu. [9]	5
I-6	<i>DIS1</i> and <i>DIS2</i> Play a Role in Tropisms in <i>Arabidopsis thaliana</i>. J.C. Reboulet, P. Kumar and J.Z. Kiss. [10]	5
I-7	Effect of Modification of Environmental Density and Buoyancy on Growth and Gravitropic Response in Maize Roots. J.L. Robbins and T.J. Mulkey. [11]	5
I-8	Humidity Control for Plant Studies in a Low Pressure Growth Chamber. J.R. Truett, R.A. Bucklin and M.J. Correll. [12]	5
I-9	Identification of Genes Involved in the Signal Transduction Pathway of Mechano-sensing in Roots. Q. Wu, M-L. Sauer, E.M. Brown, M. Cheng, L. Shamey, C. Brown and H. Sederoff. [13]	6
I-10	Characterizing the Role of PIN4 in the Red-light Inhibition of Root Elongation. W.A. Acosta, J.Z. Kiss and M.J. Correll. [14]	6
I-11	Do Magnetic Fields Affect Plant Growth and Development? C.M. Frederick and S.E. Wyatt. [15]	6

LONG PROGRAM – 2009 ANNUAL MEETING

Friday Afternoon, November 6

Poster Session I: Student Poster Competition (cont.)

14:00 – 17:00

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I-12	Optimization of the Methodology for Studying Transpiration Rates of <i>Arabidopsis</i> in Hypobarica. S. Garcia, R.A. Bucklin and M.J. Correll. [16]	6
I-13	Long Term Effects of Hypobarica on Radish Growth and Evapotranspiration. S.J. Smith, H. Gohil, R.A. Bucklin and M.J. Correll. [17]	7
I-14	Effects of Supplemental Sucrose on Tropistic Curvature in Plant Seedlings. K.M. Travis, P. Kumar and J.Z. Kiss. [18]	7
I-15	High Resolution Analysis of Gravitropic Response in the Auxin-Insensitive Mutant <i>tir1</i>. K.L. Cooper, D.R. Lewis and G.K. Muday. [19]	7
I-16	Altered Gravitropism and Auxin Transport in the <i>scd1</i> Mutant of <i>Arabidopsis thaliana</i>. J. Isley, C. Mattox, K.L. Cooper, T. Falbel and G.K. Muday. [20]	7

Friday Evening, November 6

17:00	Committee Meetings
18:00	Reception

Saturday Morning, November 7

8:00 Registration Opens

<p>Scientific Symposium II Biological Engineering</p> <p>8:30 - 12:00</p> <p><i>Marshall Porterfield, Symposium Chair</i></p>
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8:35	10
Transfer of Genes from <i>Pyrococcus furiosus</i> into Model Plants to Facilitate Stress Tolerance in Spaceflight Environments. W.F. Boss, Y.J. Im, R. Killens, A. Lee, M. Ji and A.M. Grunden. [21]	
9:20	10
Synthetic Biology and the Rational Design of Microbial Chemical Factories. K.L.J. Prather, T.S. Moon, C.H. Martin, S.H. Yoon and J.E. Dueber. [22]	
10:05	
<i>Break</i>	
10:20	10
An Implantable Biochip to Influence Outcomes in Trauma-induced Hemorrhage. A. Guiseppi-Elie. [23]	
11:05	10
Engineered Minichromosomes and Microgravity Environments. G.P. Copenhaver, S. Luo, S.R. Carlson, G.R. Rudgers, J.M. Mach, E. Grunden, P. Barone and D. Preuss. [24]	
12:00	
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13:00	12
Special Lecture: What is Required to Maintain Skeletal Muscle Health in Space? D.A. Riley and J.M. Van Dyke. [25]	

Saturday Afternoon, November 7

Concurrent Oral Sessions I and II

14:00 – 16:15

Oral Session I

Gravitational and Space Biology: Plants

Chris Brown, Chair

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14:00	The gravity persistence signal Mutants of <i>Arabidopsis thaliana</i>: Insights into Gravitropic Signal Transduction. S.E. Wyatt, B. Justus, N. George, C. Schenck, C. Bruggeman, K. Shen and D. Luesse. [26]	14
14:15	Basic Characterization of the gravity persistent signal 5 Mutant in <i>Arabidopsis thaliana</i>. D.R. Luesse, H.T. Huynh, J. Kinser and S.E. Wyatt. [27]	14
14:30	Gravity-Modulated Transporters: Molecular Candidates that Could Drive the Trans-Cell Calcium Current in Single-Celled Spores of <i>Ceratopteris richardii</i>. S.J. Roux, T. Bushart, G. Clark and D.M. Porterfield. [28]	14
14:45	Functional Characterization of a Gravity-regulated Sterol-binding Protein in <i>Arabidopsis thaliana</i> Roots. J.D. Kajla, C.S. Brown and H.W. Sederoff. [29]	14
15:00	<i>Break</i>	
15:15	Differential Regulation of plant miRNAs in Response to Gravity. S. Kumar, B. Wheeler, S. Heber, C. Brown, T. Lomax and H. Sederoff. [30]	15
15:30	Lessons Learned from TROPI-1, a Spaceflight Experiment to Study Plant Tropisms. P. Kumar, R.E. Edelmann and J.Z. Kiss. [31]	15
15:45	The Interacting Effects of CO₂ and Hypobaria on Growth and Transpiration of Radish. H.L. Gohil, R.A. Bucklin and M.J. Correll. [32]	15
16:00	The Flavonol Quercetin Regulates Root Gravitropism and Auxin Transport and Shows Auxin Induced Synthesis. D.R. Lewis, D. Cooper, K.L. Cooper and G.K. Muday. [33]	15

Saturday Afternoon, November 7

Concurrent Oral Sessions I and II (cont.)

14:00 – 16:15

Oral Session II

Biological Technologies and New Capabilities for Research and Spaceflight

Adarsh Deepak, Chair

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14:15	Spaceflight Hardware Used to Differentiate Bone Marrow Stem Cells. P.J. Duke, H. Luong, W. LeBoeuf, Q. Diep and S. Cai. [35]	18
14:30	A Novel PharmaSat Compatible Lab-on-a-chip Platform for Studying <i>Cyanobacterial</i> Gravitational Physiology. A. Ul Haque, J.C. Claussen and D.M. Porterfield. [36]	18
14:45	Real Time Physiology of Silica-entrapped Biofilms used for Water Reuse in Life Support. E.S. McLamore, D. Jaroch, J.L. Rickus, D.M. Porterfield and M.K. Banks. [37]	18
15:00	<i>Break</i>	
15:15	Conducting Gravitational Biology Research on Suborbital Commercial Flights. Y.D. Cagle and E.B. Wagner. [38]	19
15:30	Magnetic Levitation of Human A431 Cels M.J.A. Moes, J.C. Gielen, R. Bleichrodt, J.J.W.A. van Loon, P.C.M. Christianen and J. Boonstra. [39]	19
15:45	A Novel Bedrest Analog of Lunar Exploration. A.M. Hanson, A.J. Rice, S. Novotny, K.O. Genc, M. Kuklis, A. Licata and P.R. Cavanagh. [40]	19
16:00	Japanese Research Group for Animal and Human Physiology in Lunar and Martian Gravity. Y. Kumei, J.L. Zeredo, T. Yabushita, T. Ishida, S. Seki, T. Ikeda, K. Toda, M. Matsuura, Y. Nomura, K. Iwasaki, F. Kawano, Y. Ohira, M. Okuno, D. Kageyama and M. Yamashita. [41]	19

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II-2	Effects of Active Heat Shock Factor 1 on Skeletal Muscle Hypertrophy in Mice. K. Goto, Y. Ohno, A. Nakai, T. Sugiura, Y. Ohira and T. Yoshioka. [43]	22
II-3	Gene Expression Changes in Space Flown <i>Caenorhabditis elegans</i> Exposed to a Long Period of Microgravity. R. Jamal, J. Nurul-Faizah, S.M. Then, S. Nathan, N.J. Szewczyk, L.S. Stodieck and R. Harun. [44]	22
II-4	Norepinephrine Stimulates or Inhibits Contraction of Fibroblast Populated Collagen Gels Depending on Its Concentration. B. P. Johnson-Wint. [45]	22
II-5	Simulated Microgravity: a Novel Approach to Embryonic Stem Cell Culture. Y. Kawahara, T. Manabe, M. Matsumoto, T. Imura, T. Kajume, M. Takeda and L. Yuge. [46]	23
II-6	Changes in Gene Expression of HepG2 Cells Exposed to Microgravity. A.A.N. Khairul-Bariah, S.M. Then, R. Rageshwary, N. Fazlina, W.N. Wan-Zurinah, H. Roslan, D.M. Klaus, L.S. Stodieck and R. Jamal. [47]	23
II-7	Lessons Learned from TROPI-1, a Spaceflight Experiment to Study Plant <u>Tropisms</u>. P. Kumar, R.E. Edelmann and J.Z. Kiss. [48]	23
II-8	New Insights into Phototropism from Experiments in Microgravity. K.D. Millar, P. Kumar and J.Z. Kiss. [49]	23
II-9	Effect of Hypergravity Exposure on Abundance of Transcripts Associated with Free Fatty Acid Transport in Rat Mammary Gland. O.V. Patel and K. Plaut. [50]	24
II-10	The Effects of Microgravity on Thermostable T1 Lipase Protein Crystal. R.N.Z.A. Rahman, M.S.M. Ali, T.C. Leow, A.B.Salleh and M.Basri. [51]	24

Saturday Afternoon, November 7

Poster Session II (cont.)

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Poster #		Page
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II-12	Lunar Biology Requirements Development. D. Reiss-Bubenheim, N. Rayl, and R. Briggs. [53]	24
II-13	The Life and Physical Science Laboratory (LPS) and its facilities at ESA's European Space Research and Technology Centre (ESTEC) in the Netherlands. J. Krause, C. Paille, A. Dowson, L. Zuijderduijn, S. Raffestin, N. Fritz, H. Cunha, P. Raposo, J.J.W.A. van Loon. [54]	25

Saturday Evening, November 7

18:00 **Banquet and ASGSB Business Meeting**
Keynote Speaker: Mary Schweitzer, North Carolina State University
“Dinosaurs and Space”

Sunday Morning, November 8

7:00 **ASGSB Governing Board Meeting**

8:00 **Registration Desk Opens**

Scientific Symposium III

The ISS as a National Lab

8:30 - 12:30

Ken Souza, Symposium Chair

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8:35 Use of the International Space Station as a US National Laboratory. M.L. Uhran. [55]	28
9:15 International Space Station Research and Facilities for Life Sciences. J.A. Robinson, T.M. Ruttley and C.A. Evans. [56]	28
9:55 <i>Break</i>	
10:10 Discovery of Spaceflight-regulated Virulence Mechanisms in <i>Salmonella</i> and other Microbial Pathogens: Novel Approaches to Commercial Vaccine Development. C.A. Nickerson, J.W. Wilson, C.M. Ott. [57]	28
10:50 Genetic Mediators of <i>Salmonella</i> Virulence During Spaceflight in a Nematode Model. T.G. Hammond, J.L. Becker, A.L. Johnson, J.S. Hammond, M.A. Gunter, L.S. Stodieck and P.L. Allen. [58]	28
11:30 Panel Discussion. Panelists: Fei Wang, NIH; Louis Stodieck, Bioserve; Caird Rexroad, USDA, and speakers. <i>Moderator: Neal Pellis</i>	
12:30 Lunch	

Sunday Afternoon, November 8

Oral Session III

13:30 – 16:30

Oral Session III

Space Biology and Physiology: Animals, Cells and Microbes

David Tomko, Chair

Time		Page
13:30	The PharmaSat Nanosatellite Platform for Life Science Experimentation: Effects of Space Flight on Antifungal Activity Against <i>Saccharomyces cerevisiae</i>. M. Parra, D. Ly, A.J. Ricco, M.R. McGinnis and D. Niesel. [59]	30
13:45	Space Flight Alters <i>Streptococcus pneumoniae</i> Gene Expression and Virulence Activity. U. Pandya, R. Carmichael, D.A. Watson, N. Williams, K. Sato, H.E. Ray and D.W. Niesel. [60]	30
14:00	Spaceflight Effects on Gene Expression and Exotoxin A Production in <i>Pseudomonas aeruginosa</i>. B.H. Pyle, S.C. Broadaway, K. McInnerney and K. Williamson. [61]	30
14:15	Soyuz/ISS Kubik-Experiment XENOPUS: Evidence for g-Related Critical Periods in Amphibian Development. E.R. Horn and M. Gabriel. [62]	30
14:30	Early Results from Biological Studies aboard the Japanese Experiment Module “Kibo” of the ISS. M. Takaoki, N. Fujimoto, S. Ogawa and T. Nakamura. [63]	31
14:45	<i>Break</i>	
15:00	Effects of Space Flight on Mouse Granulocytic Bone Marrow Cell Populations. S.K. Chapes, M.T. Ortega, M.J. Pecaut, D.S. Gridley, V. Ferguson and L.S. Stodieck. [64]	31
15:15	Effects of Parabolic Flight on Serotonin-related Gene Expression in the Mouse Brain. M. Yoshioka, T. Yamaguchi, H. Ohta, J. Gyotoku and T. Ochiai. [65]	31

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Oral Session III (cont.)

13:30 – 16:30

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15:30	Definition of Multi-investigator Compatible Tissue Retrieval of Male and Female Reproductive Organs to Aid in Biospecimen Sharing Program Procedures for Acute Post-flight Tissue Collection. V. Gupta, L. Holets, K.F. Roby and J.S. Tash. [66]	31
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16:00	Altered Gravity Exacerbates Chromate-Induced Genotoxicity. J.P. Wise Jr., S.S. Wise, J. Wise, J. McKay, M. Browne, K. Joyce, M. Braun, C. Wise, R. Duffy, E. Estell, J. Brown, C. Gianios Jr., M. Mason, T. Shehata, D. Hammond and J.P. Wise, Sr. [68]	32

Symposium I

Closed-Loop Regenerative Life Support for Sustainable Habitation in Space and on Earth

ABSTRACTS – 2009 ANNUAL MEETING ISSUE

[1]

MELISSA: THE EUROPEAN PROJECT OF A CLOSED LIFE SUPPORT SYSTEM. Ch. Lasseur, Brunet J., D., De Weever H., Dixon M., Dussap C.G., Godia F., Mergeay M., Van Der Straeten D., Verstraete W. European Space Agency, Thermal & Environment Control, Noordwijk, The Netherlands

The MELISSA (Micro-Ecological Life Support Alternative) project was initiated in 1989. It is intended as a tool to gain understanding of closed life support, as well as the development of the technology for a future life support system for long term manned space missions, e.g. a lunar base or a mission to Mars.

The collaboration was established through a Memorandum of Understanding and is managed by ESA. It involves several independent organisations: Ghent University, EPAS, SCK, VITO (B), University of Clermont-Ferrand, SHERPA (F), University "Autonoma" of Barcelona (E), University of Guelph (CND). It is co-funded by ESA, the MELISSA partners, the Belgian, the Spanish and the Canadian authorities. The driving element of MELISSA is the production of food, water and oxygen from organic waste recycling. Inspired by the principle of an "aquatic" ecosystem, MELISSA process comprises several sub-processes, called compartments, from the anoxygenic fermentor up to the photosynthetic units (i.e. algae and higher plants). The choice of this compartmentalised structure is required by the very high level of safety requirements and justified by the need of an engineering approach and to build deterministic control strategy.

During the past 19 years of research and development, a very progressive approach has been developed to understand and control the MELISSA loop. This approach starts from the selection of processes, their characterisation and mathematical modelling, the validation of the control strategy, up to the demonstration on Earth, at pilot scale.

The project is organised in 5 phases: Basic R&D, Preliminary flight experiment, Ground & space demonstration, Terrestrial transfer, Education & communication.

[3]

SUSTAINABLE BASE CAMP SYSTEM: REQUIRED CAPABILITY FOR THE ARMY FUTURE FORCE, 2030 & BEYOND. Kurt Preston, Army Research Office, Research Triangle Park, NC

This paper begins with an exploration of the likely world environment in the year 2030, a world that will witness a resource limited and dense urban populations struggling to secure good governance while fighting intermixed radicalized sub-elements. It argues that forward operating bases and the capability to engineer sustainable operations through the long fight are key centers of gravity. The vision is a campaign quality expeditionary force supporting full spectrum operations within the joint, interagency, intergovernmental, and multinational (JIIM) environment. To advance this vision, the Army must develop a system of systems approach to forward operating bases. The paper explores the context of how the base camps influence the operational framework and concludes by examine the bottom line question of what needs to be done now. It concludes with an examination of linkages to base camp needs in other extreme environments.

[2]

CEEF: CLOSED ECOLOGY EXPERIMENT FACILITIES. Y. Tako¹, R. Arai¹, S. Tsuga¹, O. Komatsubara¹, T. Masuda¹, S. Nozoe¹, K. Nitta². ¹Institute for Environmental Sciences, and ²Technical advisor, Institute for Environmental Sciences, Japan.

Since 2005 to 2007, material circulation has been demonstrated connecting the Closed Plant Experiment Facility (CPEF) and the Closed Animal and Human habitation Experiment Facility (CAHEF) of the Closed Ecology Experiment Facilities (CEEF). The CPEF has a Plant Cultivation Module (PCM), which comprises of three plant chambers illuminated solely by artificial lighting, one plant chamber illuminated by both natural and artificial lighting, a space for preparation, and an airlock, and a physical/chemical material circulation system. The total plant cultivation area of the PCM was 150 m². The CAHEF has an Animal and Human habitation Module (AHM), which comprised of an animal room, a habitation room, closed corridor, and an airlock, and a physical/chemical material circulation system. During the material circulation experiments, two humans (called as eco-nauts) stayed in the CEEF, and we called it closed habitation experiment. In these experiments, 23 crops including rice, soybean, peanut, and sugar beet were cultivated in the PCM, and two goats stayed in the AHM. Almost all of the food consumed by the eco-nauts and the feed to the goats were produced from crops in the PCM. The oxygen added to the atmosphere of the PCM by photosynthesis of crops was separated and supplied to the atmosphere of the AHM. Increased carbon dioxide in the AHM atmosphere by respiration of eco-nauts and goats was separated and supplied back to atmosphere of the PCM. In addition to food production and circulation of air constituents, water circulation was also conducted in the CEEF in 2006 and 2007. In addition to them, waste processing and circulation of materials from the waste in the CEEF were also conducted in 2007. Closed habitation experiments in 2005, each lasting one week, were conducted three times. In 2006, although the eco-nauts changed by week, 2-week habitation was conducted three times. In 2007, 1-week, 2-week (two times) and 4-week habitation were conducted. Data obtained in all of above experiments conducted in 2005-2007 will be also invaluable for examination and planning of human-in-loop systems necessary for independent long-term human living habitats such as lunar or Martian base.

(This research was conducted under contract with Aomori Prefecture.)

[4]

THE PAST, PRESENT, AND FUTURE OF BIOGENERATIVE LIFE SUPPORT FOR SPACE HABITATION. R.M. Wheeler, NASA Sustainable Systems Division, Kennedy Space Center, FL.

Bioregenerative life support systems have been discussed since the works of Tsiolkovsky in early 20th century. Central to the concept is the use of photosynthetic organisms and light (energy) to regenerate air and food. Research in the area expanded rapidly 1950s and 60s through the works of Jack Myers and others, and focused largely on algal systems for O₂ production and CO₂ reduction. The testing expanded somewhat to include higher plants, and even some space flight experiments by Herb Ward and colleagues in the 1960s. Through much of the 1970s and 1980s, the Russian BIOS projects with Josef Gitelson and colleagues in Krasnoyarsk set the standard for bioregenerative life support research, with fully-integrated tests of human crews lasting up to several months. NASA had no comparable effort but initiated its Controlled Ecological Life Support Systems (CELSS) Program ca. 1980, which focused largely on higher plant testing at various universities and NASA's Ames Research Center. At about the same time, similar efforts started in Cadarache, France and the National Aerospace Laboratory in Tokyo. In the late 80s and 90s, findings from NASA's university research were used to conduct breadboard testing at Kennedy Space Center using a 20 m² area in 113 m³ atmospherically closed chamber. Related tests were also conducted in the 1990s at Johnson Space Center, and a more fully integrated test bed called BIO-Plex was planned, but never completed. The European Space Agency's MELISSA Project and the Japanese CEEF Project expanded their research through the 1990s and reports from both these efforts will be presented at this meeting. NASA has currently stopped its bioregenerative research but other space agencies around the world are maintaining active programs. A likely scenario for implementing bioregenerative life support might start with a small plant growth unit or salad machine to produce some fresh foods for long duration lunar outposts, which could be expanded over time. This lunar testing could also assess "Mars-forward" concepts and risks for longer duration Mars missions, where bioregenerative life support will play a more crucial role.

Poster Session I

Student Poster Competition

[5]

A HIGHLY SENSITIVE NANOCUBE AUGMENTED CARBON NANOTUBE-BASED LAB-ON-A-CHIP PLATFORM FOR CONTINUOUS ASTRONAUT HEALTH MONITORING .

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Space travel presents a myriad of health concerns for astronauts. In addition to bone and muscle degradation, low gravity environments can adversely affect metabolism and release of hormones. These health challenges are even greater concern in long duration space missions such as missions to Mars or lunar base stations. Therefore it is of great importance to develop technologies that can continuously monitor the health of astronauts on such missions but at the same time have minimum impact on mission payload. To this end we present a carbon nanotube (CNT) based electrochemical biosensor capable of non-invasive and continuous monitoring of key biomarkers of stress and metabolism alterations: namely lactose and glucose. This highly sensitive biosensor consists of single-walled (SWCNT) carbon nanotubes decorated with Au/Pd nanocubes. We have demonstrated glucose measurements at a detection limit of 1.3 μM (S/N = 3) and linear sensing range spanning from 10 μM to 50 mM. The sensitivity of the Au/Pd SWCNT biosensor not only surpasses the capability of similar CNT-based biosensor, it opens the door towards the early detection of even the slightest changes in clinically important human biomarkers. We expect to integrate the Au/Pd-SWCNT biosensor onto a lab-on-a-chip platform for efficient multiplexed analyte sensing that can be interfaced with a handheld portable reader and data logger for real-time sensing. With integrated working and reference electrodes, lactose and glucose, two biomarkers associated with stress and metabolism will be simultaneously monitored by chemically functionalizing distinct Au/Pd electrodes with the oxidase enzymes associated with the respective biomarkers. We expect this sensor to revolutionize astronaut health monitoring by enabling low cost diagnostics upon which optimized administration of counter measures can be performed.

[6]

NONINVASIVE MEASUREMENT OF ROOT AUXIN FLUX USING A SELF-REFERENCING IAA MICROSENSOR

Alfred Diggs^{1,2,3}, Eric McLamore^{1,2,4}, Percy Calvo Marzal^{1,5}, Jin Shi^{1,2,6}, Jonathan Claussen^{1,2,3}, Angus Murphy^{1,6}, Marshall Porterfield^{1,2,3,5,6,7}

¹Purdue University ²Bindley Bioscience Center: Physiological Sensing Facility ³Department of Agricultural and Biological Engineering ⁴School of Civil Engineering ⁵Department of Chemistry ⁶Department of Horticulture & Landscape Architecture ⁷Weldon School of Biomedical Engineering

The phytohormone, indole acetic acid, is essential for the regulation of plant growth and development, and plays a role in cell signaling. For example, IAA is an important factor of gravity directed plant organ growth, or gravitropism. Due to the key role IAA transport plays in plant physiology there is a significant need for methods to detect and study this key regulatory compound. We showcase here new electro-analytical sensor approaches for non-invasive measurement of IAA flux/transport in plant roots. Using an optimized sensor design we fabricated a platinum black carbon nanotube amperometric microsensor (2-4 μm tip diameter) that is selective and capable of measuring IAA flux using self-referencing (SR) sensor technique. The SR technique is a sensing modality based on Fick's first law of diffusion for characterizing diffusive analyte transport within the unstirred layer. This sensor was validated by using an artificial diffusion gradient and then by noninvasively monitoring real time root induced IAA fluxes of inbred lines and IAA transport mutants of *Zea mays*. In addition, we were able to detect endogenous transient IAA flux within the mass boundary layer surrounding both the inbred line and mutant plant roots. This sensing modality eventually could be used to measure IAA flux of wild type plants during gravitropism as well as screen for auxin transport mutants.

[7]

THE ROLE OF *DREB2B* IN GRAVITROPISM AND PHOTOTROPISM

C. Johnson, P. Kumar, J.Z. Kiss
 Department of Botany, Miami University, Oxford, Ohio

DREB2B is a Dehydration Response Element that is located on chromosome 3 in *Arabidopsis thaliana* and is known to code for proteins that initiate a response to dehydration and salt stress. Dehydration Response Elements (DREs) like *DREB2* are placed within the AP2/EREBP gene family which functions in stress response in plants. Results from the gene profiling experiments of plants grown in microgravity on the International Space Station during the TROP1 project indicated up-regulation of the *DREB2* genes in long-term exposure to microgravity. This current study tests the role of *DREB2B* in gravitropism and phototropism by comparing the Columbia wild type with *dreb2b* mutants in a series of ground-based studies with seedlings and inflorescence stems. Preliminary results of studies with tropisms with a high resolution feed back system show that the mutant roots have an attenuated response relative to wild type roots. Our studies are significant because EREBP/AP2 gene family has not yet been implicated in tropistic response.

(Supported by NASA grant NCC 2-1200.)

[8]

STRATEGIES FOR CLONING OF THE *GRAVITY PERSISTENCE SIGNAL (GPS)* GENES. B. Justus¹, N.M. George¹, C. Schenck¹, C. Bruggeman¹, D.R. Luesse² and S.E. Wyatt¹

¹Dept. of Environmental & Plant Biology, Ohio University, Athens, OH and ²Dept. of Biological Sciences, Southern Illinois University, Edwardsville, IL

GPS mutants are a class of *Arabidopsis* mutants potentially defective in the signal transduction events after gravi-stimulation. When stimulated at 4°C, statolith sedimentation occurs, but auxin transport is impaired. Our lab aims at identifying the genes altered in these mutants and thereby understanding the function of these genes in the process of gravitropism. The GPS mutants were identified from the T-DNA tagged population developed by Ken Feldmann and available at the *Arabidopsis* Biological Resource Center. PCR based methods such as Thermal Asymmetric Interlaced (TAIL) PCR, inverse PCR and adaptor ligation methods have been used to identify the flanking regions of the T-DNA insertion. Using TAIL PCR, *GPS1* and *GPS3* were identified. *GPS4* and *GPS5* were discovered by an adaptor ligation method. *GPS2* and *GPS6* have yet to be identified. Although *gps2* is no longer Kanamycin resistant, there is evidence that a part of the tag remains intact. The T-DNA tags in three of these mutants seem to have extended regions integrated beyond the canonical left border. PCR results also indicate a possibility of tandem tags. Hybridization based methods using the left border sequence as probe and modified PCR approaches are being adopted to clone these genes. (Partially funded by NSF:0618506)

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[9]

TRANSPLANTATION OF BONE MARROW STROMAL CELLS CULTURED UNDER SIMULATED MICROGRAVITY INTO A SPINAL CORD INJURY RAT. M. Takeda¹, T. Magaki¹, A. Sasaki², T. Manabe², M. Matsumoto², Y. Kawahara², L. Yuge², and K. Kurisu¹.

¹Department of Neurosurgery, Graduate School of Biomedical Sciences, and ²Division of Bio-Environmental Adaptation Sciences, Graduate School of Health Sciences, Hiroshima University, Hiroshima, Japan.

Recently, regenerative medicine has gained significant attention for the treatment of central nervous system diseases. Although bone marrow stromal cells (BMSCs) are considered to have a high engraftment potential, problems such as decline in their functional potency due to in vitro culturing have been reported. Here, we investigated the activity of rat BMSCs (rBMSCs) under simulated microgravity conditions, with special focus on transplantation into a rat. rBMSCs cultured under simulated microgravity condition indicated Oct-4 and CXCR4 in contrast to those cultured under 1G condition. Therefore, rBMSCs cultured under simulated microgravity were considered to possess an undifferentiated state and high migration ability. Next, we intravenously injected cells in a rat with spinal contusion. After 3 weeks, graft cells were identified in the damaged spinal region. Graft rBMSCs cultured under microgravity exhibited greater survival on the periphery of the lesion, and the motor function of the grafted rat improved significantly. In addition, a high expression level of apoptosis inhibiting factor on the periphery of the lesion was observed. Our study indicates that culturing rBMSCs under simulated microgravity enhanced migration ability and improved motor function of a grafted spinal cord injury rat. As a result, transplantation of simulated microgravity exposed rBMSCs may facilitate functional recovery from spinal cord injury by neuroprotective action.

[11]

EFFECT OF MODIFICATION OF ENVIRONMENTAL DENSITY AND BUOYANCY ON GROWTH AND GRAVITROPIC RESPONSE IN MAIZE ROOTS. J.L. Robbins & T.J. Mulkey, Dept. of Biology, Indiana State University, Terre Haute, IN.

The mechanism by which plants sense gravity is not fully understood. The hydrostatic model of gravitropic sensing states that the gravity sensor in a plant cell is the entire protoplast, or contents of the cell. Hence, gravitropic sensing depends on the buoyancy of the protoplast relative to the density of its external environment. Primary roots of maize (Federal Hybrid RK112-1, Elgin, IA), with a length of about 1 cm, were placed in environments of various densities using air, oxygenated water, sucrose/polyethylene glycol (PEG), and Ficoll 400.

The rates of growth and gravitropic curvature were monitored using time-lapse video and digital recordings. Roots grown in air showed growth rates comparable to roots grown in oxygenated water, but the roots grown in air showed greater gravitropic curvatures compared to roots grown in the more dense water medium. In initial studies, solutions containing only sucrose proved unsuitable, because they produced erratic gravitropic responses in roots. In subsequent studies, the sucrose was used in conjunction with polyethylene glycol (PEG) or the sucrose was replaced with Ficoll PM 400. Roots show no significant curvature in a 10% sucrose solution with 5% polyethylene glycol (PEG). Elongation of the roots is inhibited for approximately 2 hrs, but the elongation rate recovers to within 10% of the control rate. In experiments, Ficoll did not inhibit the elongation of roots. Roots grown in the higher density Ficoll solutions showed decreased gravitropic curvature. The decrease in gravitropic curvature with increasing medium density did not appear to involve statoliths; altering the environmental density did not alter sedimentation of statoliths within the root tip. When the density of the external media was changed, it produced a change in the gravitropic response of roots.

[10]

DIS1 AND DIS2 PLAY A ROLE IN TROPISMS IN ARABIDOPSIS THALIANA. James C. Reboulet, P. Kumar, J.Z. Kiss
Dept. of Botany, Miami University, Oxford, OH.

To better understand the role of the cytoskeleton in tropisms, we performed studies of gravitropism and phototropism with seedlings of *distorted1* (*dis1*) and *distorted2* (*dis2*) mutants of *Arabidopsis thaliana* which are defective in the ARP (actin related protein) 2/3 complex. The aim of this investigation was to test the hypothesis that this actin-binding protein family is involved in mechanisms of tropisms in plants. In general, we found that *DIS1* had a greater effect on tropisms compared to *DIS2*. *DIS1* enhanced gravitropism in roots of dark-grown seedlings and in inflorescence stems while *DIS2* also enhanced gravitropic responses in inflorescence stems. In contrast, in blue-light-based phototropism, *DIS1* attenuated the response in hypocotyls of dark-grown seedlings and in red-light-based positive phototropism in roots. Taken together, these studies are the first to suggest that the ARP 2/3 complex, a major family of actin-binding proteins, is involved in the pathways of gravitropism and phototropism.

(Supported by NASA: NCC2-1200)

[12]

HUMIDITY CONTROL FOR PLANT STUDIES IN A LOW PRESSURE GROWTH CHAMBER. J.R. Truett, R.A. Bucklin, and M.J. Correll, Dept. of Agricultural and Biological Engineering, University of Florida, Gainesville, FL.

Plants grown for Advanced Life Support Systems (ALS) for missions to the Moon or Mars will likely be grown in low pressure environments (i.e. hypobaria). Despite this, very few studies have looked at the effects of the gas phase, particularly relative humidity, on plant growth in hypobaria. Therefore, to study the interacting effects of humidity and hypobaria on plant growth a humidity control system was developed for a low pressure growth chamber. This was achieved through the use of a cooling coil, an insulated vessel in which the coil was located, and a fan. The cooling coil was maintained at a desired temperature by running chilled water through it from an external chiller unit (WK 1200, Lauda, Germany). Water saturated in the air at a given temperature condensed on the surface of the coil and was collected to recycle back to the plant. The cooling coil was able to condense 0.002685 grams of water per second at one atmosphere at a temperature of 28.37°C with a coil temperature of 10°C until the coil reached saturation. The coil maintained the relative humidity when set at a given temperature, regardless of the atmospheric pressure. This system for controlling humidity in an enclosed chamber is low cost, easy to assemble, and compact. Studies using this system indicated that the plants grown in flasks at low relative humidity (60% RH) and low pressure (33 kPa total pressure at 25°C with 100Pa CO₂ and 21kPa O₂) showed no signs of desiccation. This suggests that plants are able to reduce water loss in low humidity, low pressure environments.

(Supported by UCF-UF Space Grant Consortium 2005)

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[13]

IDENTIFICATION OF GENES INVOLVED IN THE SIGNAL TRANSDUCTION PATHWAY OF MECHANO-SENSING IN ROOTS. Q. Wu, M-L Sauer, E. M. Brown, M. Cheng, L. Shamey, C. Brown and H. Sederoff. Dept. of Plant Biology, NC State University, Raleigh, NC.

Root growth is regulated by environmental and genetic components. We are identifying the genetic basis and the signal transduction/response pathway that regulates the roots' ability to sense mechanical impedance. Roots that encounter an impenetrable surface exhibit a growth pattern that seems to balance between responses to gravity and mechanical stimulation (Massa and Gilroy, 2003). Microarray analysis showed that gravitropic and mechanical stimulation have both common and stress-specific pathways regulating gene expression (Kimbrough et al., 2004). We have developed a screen using *Arabidopsis* T-DNA insertion lines to identify mutant plants that are able to penetrate high-density agar as a first selection criterion. For wild type plants, 3.2% agar medium impedes root growth, while the selected mutant lines can easily penetrate this medium. Using TAIL-PCR we have identified genes that are likely to be involved in the roots' ability to overcome mechanical impedance. (Funded by NASA grant NNX08AR20G, NC Space Grant, and NSF REU Synthetic Biology).

Kimbrough JM, Salinas-Mondragon R, Boss WF, Brown CS, Sederoff HW (2004) The Fast and Transient Transcriptional Network of Gravity and Mechanical Stimulation in the *Arabidopsis* Root Apex. *Plant Physiol.* **136**: 2790-2805

Massa GD, Gilroy S (2003) Touch and gravitropic set-point angle interact to modulate gravitropic growth in roots. *Advances in Space Research.* **31(10)**: 2195-2202

[15]

DO MAGNETIC FIELDS AFFECT PLANT GROWTH AND DEVELOPMENT? C.M. Frederick, S.E. Wyatt. Department of Environmental and Plant Biology, Ohio University, Athens, Ohio

Earth's magnetic field strength is 30,000 nanotesla at the surface, whereas at the surface of Mars, the strength of the magnetosphere is 1,000 nanotesla. If/when mankind colonizes Mars, what effect might the reduced magnetosphere have on growth and development? How will biological processes be sustained in a reduced magnetic field? How can plants be utilized as indicators of orientation in a magnetic field? To test the effect of plant growth in a magnetic field, one magnet (6.4mm x 24mm) was affixed to MS plates. Seeds of WT were plated either adjacent to the magnets in the low-G negative field or on plates without magnets, and the plates were clino-rotated to eliminate the effects of gravity. After seven days, total seedling length was recorded along with hypocotyl bending. The aquatic bacterium *Magnetospirillum magnetotacticum* biomineralize magnetite for migration to preferred oxygen gradients (magnetotaxis). The bacterial gene COG3536, is responsible for magnetosome development, iron oxide and iron sulfide nucleation, as well as nanocrystal synthesis. At3g27340, an *Arabidopsis thaliana* gene that encodes a protein of unknown function, has a similar protein sequence to COG3536. T-COFFEE was used to align multiple sequences and similar conservation was found in *Vitis vinifera* and *Oryza sativa*. At3g27340 is located on the third chromosome of *Arabidopsis thaliana*, and this gene is expressed during 15 growth stages and in 23 plant structures including the male gametophyte, pollen tube, seed, embryo, hypocotyl, leaf flower, stamen, carpel, sepal, petal and inflorescence. Two T-DNA insertion mutants, SALK_011817 and SALK_062099, have been selected for study. Continued research includes the characterization and rescue of the phenotype of the mutants. Partial funding provided by ASPB SURF award and the Jeanette Graselli-Brown Undergraduate Research Award to CMF.

[14]

CHARACTERIZING THE ROLE OF PIN4 IN THE RED-LIGHT INHIBITION OF ROOT ELONGATION. W.A. Acosta¹, J.Z. Kiss² and M.J. Correll¹

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The identification of key genes involved in red-light-signaling pathway in plants will suggest mechanisms that plants use to adjust their growth in response to light. Roots have been shown to display positive phototropism and a reduced rate of elongation when exposed to periods of red light. The role of auxin transporters in these responses is unknown. Microarray data suggests that gene expression of an auxin transporter, PINOID4 (PIN4), is involved in the red-light inhibition of root elongation. Gene expression studies using microarrays were performed with the roots of phytochrome mutants *phyA*, *phyB*, and *phyAB* and wild-type (Ler) exposed to 1h red light and compared to dark controls. Interestingly, only six genes were commonly upregulated in all plants and none were commonly downregulated for all plants. The common upregulated genes include ATROPGEF12/MEE64/ROPGEF12 (MATERNAL EFFECT EMBRYO ARREST 64; AT1G79860), LHB1B1 (Photosystem II light harvesting complex gene 1.4; AT2G34430), LHCB4.2 (LIGHT HARVESTING COMPLEX PSII; AT3G08940), UGT84A2 (UDP-glycosyltransferase/sinapate 1-glucosyltransferase; AT3G21560), ELIP1 (EARLY LIGHT-INDUCIBLE PROTEIN; AT3G22840), and ELIP2 (EARLY LIGHT-INDUCIBLE PROTEIN 2; AT4G14690). These genes are primarily associated with chlorophyll development which is known to occur for root growth in red light. As for auxin transporters, PIN4 expression was primarily controlled by *phyA* in roots exposed to red light since the expression levels were upregulated in Ler and *phyB* but not in *phyAB* compared to dark controls. Studies on the elongation rates of PIN4 roots in red light and confirmation of the microarray data with qRT-PCR are currently being performed. Taken together, it appears that red light induces the expression of PIN4 through phytochrome A and this may result in altered translocation of auxin thus decreased elongation of roots in red light. (Supported provided by HHMI: Science for Life Program at UF and NASA: NCC2-1200)

[16]

OPTIMIZATION OF THE METHODOLOGY FOR STUDYING TRANSPIRATION RATES OF ARABIDOPSIS IN HYPOBARIA. S.

Garcia, R.A. Bucklin and M.J. Correll

Dept. of Agricultural and Biological Engineering, University of Florida, Gainesville, FL.

Plants grown for Advanced Life Support on Mars or the Moon will be grown at lower pressures (i.e., hypobaria) to reduce gas leakage from the growth chambers, to maintain structural integrity of the chambers, and to minimize the use of valuable gases. Unfortunately, plants grown in hypobaria can have enhanced rates of transpiration compared to plants grown at higher pressures. This can result in desiccation of the plant if water is not readily available. Therefore, an understanding of transpiration rates of plants in hypobaria is required to develop engineering controls to prevent the desiccation of plants. Since *Arabidopsis* is an important model organism for plant studies both on Earth and in Space, we have developed a system for measuring evapotranspiration and transpiration rates in *Arabidopsis*. *Arabidopsis* seedlings were grown hydroponically using Gibeau's solution, exchanging EDDHA for EDTA. The seeds were placed on rock wool plugs in bottomless microfuge tubes that were inserted into larger tubes to prevent root entanglement and were floated on the solution with a foam board which allowed for even hydration. The container was placed in an aluminum box with an 8hr photoperiod provided by cool-white fluorescent lights (150 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The shorter photoperiod delayed flowering and increased leaf biomass. However, preliminary data indicated that the leaf surface area was too small to sufficiently measure transpiration rates due to limited leaf growth. In addition, plants had thin stems that were elongated suggesting that the red wavelength of light was not sufficient to inhibit stem elongation. Further studies are underway that increase light intensity and supplement the lighting with red wavelengths to promote leaf expansion and shorter plants that can be used for transpiration studies in hypobaria. Studies on the transpiration rates of plants in hypobaria are the first steps towards developing decision support tools for water management for crop growth for long-term space exploration. (Support was provided by UCF-UF Space Grant Consortium 2005).

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[17]

LONG TERM EFFECTS OF HYPOBARIA ON RADISH GROWTH AND EVAPOTRANSPIRATION. S.J. Smith, H. Gohil, R.A. Bucklin, and M.J. Correll Department of Agricultural Engineering, University of Florida, Gainesville, FL 32611.

Plants can grow in hypobaric conditions provided that the partial pressures of CO₂, H₂O, and O₂ levels are maintained at adequate levels for plant growth. This allows for the possibility of growing plants in low pressure (i.e., hypobaria) greenhouses on Mars or the Moon. However, the interacting effects of the gas phase and low total pressure on plant germination, growth, transpiration, and photosynthesis for long term is unclear. Therefore, we tested methods for growing radish (cv. Cherry Belle) long term in hypobaria using a hydroponic flask system or Rockwool cubes. The flask system consisted of a wicking system that hydrated the seedlings and the Rockwool cube system had nutrients pumped to the cube during growth. For the flask system, the radish seed was rolled in brown germination paper and suspended by a stopper in the nutrient solution. The flask system was useful for relatively short studies (5 days) on germination but resulted in high mortality rates (66%) due to drying of wicks under low pressure or due to high fungal contamination rates for longer term experiments (>1 week). Germination of seedlings at a pressure of 33 kPa in the shake flask at different pO₂ was 18%, 90%, and 100% at pO₂ of 4 kPa, 10 kPa, and 21 kPa, respectively. The Rockwool cubes had higher rates of survival and therefore have been chosen to grow plants for long term. Currently, measurements of photosynthetic rate, shoot height, crop yield, rates of evapotranspiration, nutrient levels, dry weight mass, fresh weight mass, and root to shoot ratios at various total pressures (100 kPa, 66 kPa, and 33kPa) with partial pressures of pCO₂ at 100 Pa, pO₂ at 21 kPa, and pH₂O at 2.2 kPa are being compared for long term studies. These studies are the preliminary steps towards developing decision support tools to manage limiting resources (i.e. water, nutrients, gases, etc.) for crops grown on long term space missions and will allow for the prediction of crop yields for Advanced Life Support. Funding provided by the University of Florida and the University Scholars Program.

[18]

EFFECTS OF SUPPLEMENTAL SUCROSE ON TROPIC CURVATURE IN PLANT SEEDLINGS. K.M. Travis, P. Kumar and J.Z. Kiss. Botany Dept., Miami Univ. Oxford, OH 45056

The interaction among tropisms is important in determining the final growth form of a plant. We have developed a project to study the interaction between gravitropism and phototropism in the plant *Arabidopsis* on the International Space Station (ISS). These experiments were termed TROPI, for tropisms, and were performed during Increment 14 on the ISS in the EMCS (European Modular Cultivation System). One technical problem with TROPI was low seed germination (15-60%) compared to approximately 90% germination on the ground. One likely cause of the low seed germination is the presence of sucrose in the growth medium which crystallized in space during the extended storage period prior to initiation of the experiments. Thus, in future projects, we would consider growing the seedlings without sucrose in the growth medium. The purpose of this project is to obtain accurate information from ground studies to be used as a baseline for the future spaceflight studies. Our results demonstrate that seedlings developed tropistic curvature in the absence of sucrose, but that phototropic curvature in roots was of a reduced magnitude in plants grown without supplemental sucrose. Current studies include an examination of both gravitropic and phototropic curvature in roots and shoots.

(Supported by NASA grant NNX09AF11G and the Miami Univ. Summer Scholar Program.)

[19]

HIGH RESOLUTION ANALYSIS OF GRAVITROPIC RESPONSE IN THE AUXIN-INSENSITIVE MUTANT *TIR1*.

K.L. Cooper¹, D.R. Lewis¹ and G.K. Muday¹ Dept. of Biology, Wake Forest Univ., Winston-Salem, NC.

When seedlings are reoriented relative to gravity, the resulting downward root growth requires asymmetric redistribution of auxin across the root tip. We are exploring the signaling pathways that control the differential growth in response to this auxin asymmetry. The TIR1 protein encodes an auxin receptor that is required for auxin responsive root growth and gene expression. Surprisingly, the *tir1* mutant of *Arabidopsis thaliana* exhibits only a slight reduction in the gravitropic response. To ask whether gravitropism is regulated by other members of the TIR1 gene family (AFB1-3), high resolution gravitropic analyses will be conducted with *tir1* and *afb1-afb3* mutants. We have also been examining expression of IAA responsive genes, including genes that encode flavonoid biosynthetic enzymes, which produce inhibitors of auxin transport. The *tir1* mutant showing loss of IAA induced flavonoid gene expression and we are exploring the role of AFB1-3 in this process. While previous research has primarily focused on the signaling and transport of the most abundant endogenous auxin, IAA, it is not clear whether a second naturally occurring auxin, IBA, regulates expression of these genes and the gravitropic response. Gene expression analyses will be conducted utilizing quantitative Real Time PCR to determine whether treatment with IBA, along with IAA, induces the accumulation of transcripts encoding flavonoid biosynthetic enzymes and whether this induction requires activity of TIR1 and AFB1-3. Finally, flavonoid accumulation will be measured directly in these mutants by confocal microscopy with a flavonol specific stain. These experiments will provide insight into the mechanisms by which auxin receptors control gravitropism and gene expression and the role of IBA in this response.

(Supported by ASPB SURF to KLC and the NSF Arabidopsis 2010 program Grant # 0820717 to GKM).

[20]

ALTERED GRAVITROPISM AND AUXIN TRANSPORT IN THE *SCD1* MUTANT OF *ARABIDOPSIS THALIANA*.

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We asked if IAA transport, the distribution of IAA efflux proteins, and dependent physiological processes are altered in *scd1* mutants, which have defects in a gene encoding a protein predicted to function in RAB-dependent protein trafficking. At the nonpermissive temperature of 25°C, *scd1-1*, a temperature sensitive allele, exhibited a reduced root elongation rate and delayed gravitropic curvature. These phenotypes were reversed at the permissive temperature of 18°C. Basipetal IAA transport from the site of gravity perception to the elongation zone, which regulates differential growth, and acropetal transport from the shoot into the root, a primary source of auxin for lateral root development, were reduced in the *scd1-1* mutant relative to wild-type at the nonpermissive temperature. We have used laser scanning confocal microscopy to examine the tissue level and sub-cellular distribution of two auxin efflux carriers, PIN1 and PIN2 fused to GFP. When *scd1-1* was grown at the nonpermissive temperature, the expression of these reporters was restricted to fewer cells, reduced in intensity, and accumulated in endomembrane structures. Together these experiments suggest that SCD1 may play an important role in targeting IAA transport proteins to the plasma membrane and in specifying auxin transport polarity, thereby regulating root gravitropism. (This work is supported by NASA grant NNX09AK82G to GKM)

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Symposium II

Biological Engineering

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[21]

TRANSFER OF GENES FROM *PYROCOCCLUS FURIOSUS* INTO MODEL PLANTS TO FACILITATE STRESS TOLERANCE IN SPACEFLIGHT ENVIRONMENTS. Wendy F. Boss¹, Yang Ju Im¹, Rushyannah Killens², Alice Lee², Mikyoung Ji², and Amy M. Grunden².
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Reactive oxygen species (ROS) are important signal molecules in plant responses to stress. However, studying the short-lived ROS signals *in vivo* is difficult and the downstream effectors of ROS such as cytosolic superoxide (O_2^-) are not well characterized. Our approach was to selectively remove O_2^- and to use comparative biology to reveal downstream events normally mediated by the ROS signal. Although plants, like other aerobic organisms, have superoxide dismutase (SOD) to remove O_2^- and prevent the production and buildup of toxic free radicals, the dismutase reaction produces oxygen, which can serve as an additional source of ROS. Unlike SOD, superoxide reductase (SOR), an enzyme present in extremophilic Archaea has a lower K_m for O_2^- than SOD and reduces O_2^- without producing oxygen. Therefore, we chose a synthetic approach using *Pyrococcus furiosus* SOR to reduce cytosolic ROS. Our hypothesis was that expressing the *P. furiosus* SOR in planta would provide a more effective ROS reducing system. We found that *P. furiosus* SOR can be produced as a soluble protein fused with GFP in planta and that plants producing GFP-SOR have enhanced tolerance to heat, light and chemically-induced ROS. Stress tolerance in the GFP-SOR producing plants correlates positively with a delayed increase in ROS-sensitive transcripts and a decrease in ascorbate peroxidase activity. The SOR plants provide a good model system to study the impact of cytosolic O_2^- on downstream signaling events in plant growth and development and hold promise as a means for improving stress tolerance in crop plants and other organisms. (This work was supported by grants from NIAC and the USDA to AMG and WFB.)

[22]

SYNTHETIC BIOLOGY AND THE RATIONAL DESIGN OF MICROBIAL CHEMICAL FACTORIES. K.L.J. Prather¹, T.S. Moon¹, C.H. Martin¹, S.H. Yoon¹, and J.E. Dueber². ¹Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA. ²California Institute for California Institute for Quantitative Biomedical Research and Berkeley Center for Synthetic Biology, Berkeley, CA.

The objective of synthetic biology is simply stated: to make biology easier to engineer. In principle and practice, this goal includes efforts to create and characterize standardized biological parts, to design new biological systems, to re-design existing biological systems, and to develop the computational tools and models to facilitate these efforts. The potential applications of synthetic biology are far-ranging, from bio-computing and bio-memory to the design and synthesis of novel therapeutics, and perhaps for space and gravitational biology. One particularly active area of synthetic biology involves the use of microbes for the specialized production of small molecules. These activities build upon the tools and techniques developed within the discipline of metabolic engineering, which has led to productive systems at scales ranging from the sub-milliliter (e.g., in 96-well plates) to many thousands of liters (e.g., in large scale fermentations).

Our group is interested in applying the principles of metabolic engineering and synthetic biology towards the design and construction of novel biosynthetic pathways for specified target compounds. In particular, we focus on the production of molecules with either no known biological routes or with biosynthetic pathways that are not readily amenable towards assembly in microbial hosts. Using this approach, we have achieved the microbial synthesis of glucaric acid (a dicarboxylic acid) and a variety of hydroxy-acids as value-added compounds from biomass. In the process, we have exploited enzymes as interchangeable parts and have applied novel devices towards the improvement of these pathways. We have also encountered challenges with constructing productive organisms in a predictive manner through the use of well-characterized biological parts.

[23]

AN IMPLANTABLE BIOCHIP TO INFLUENCE OUTCOMES IN TRAUMA-INDUCED HEMORRHAGE. A. Guiseppi-Elie, ¹Center for Bioelectronics, Biosensors and Biochips (C3B), Clemson University, 100 Technology Drive, Anderson, SC 29625.

A temporary, implantable, integrated glucose and lactate biosensor and communications biochip for physiological status monitoring during hemorrhage is being developed. Lactate levels correlate with the severity of injury and are a surrogate of oxygen debt. Post traumatic injury includes hyperglycemia, with continuously elevated glucose levels leading to extensive tissue damage, septicemia and multiple organ failure. Design, microfabricate and test a biotransducer based on the microdisc electrode array as well as develop an encapsulating hydrogels that mitigates fibrous capsule formation. Conduct *in vitro* studies using cell lines to evaluate cytotoxicity. Conduct *in vivo* studies in a *Sprague Dawley* hemorrhage model.

Biorecognition layers, 1.0 – 5.0 microns thick, of p(HEMA-co-PEGMA-co-HMMA-co-MPC) hydrogels show diffusion coefficients for the redox mediator, ferrocene monocarboxylic acid (FcCOOH), that ranged from $2.64 \times 10^{-8} \text{ cm}^2/\text{s}$ (1 M% TEGDA) to $4.87 \times 10^{-9} \text{ cm}^2/\text{s}$ (12 M% TEGDA). Electropolymerization of pyrrole within the hydrogel resulted in a membrane co-network [p(HEMA-co-PEGMA-co-HMMA-co-MPC)-PPy] which conferred interference screening against the endogenous interferents. *In vitro* cell proliferation and viability studies confirmed these polymers non-cytotoxic. The glucose biosensor exhibited a dynamic linear range of 0.10 - 13.0 mM glucose with a response time (t_{95}) of 50 s. The immobilized glucose oxidase within the hydrogel membrane yielded a $K_{m(\text{app})}$ of 35 mM. Linearity for assayed lactate was up to 90 mM, which represents a 30-fold increase in linear dynamic range compared to the use of planar electrodes. Operational stability gave 80% of the initial biosensor response after 5 days.

An implantable biochip that supports telemetric reporting of intramuscular lactate and glucose levels allows development of resuscitation approaches for civilian and combat trauma victims.

[24]

ENGINEERED MINICHROMOSOMES AND MICROGRAVITY ENVIRONMENTS

Gregory P. Copenhaver^{1*}, Song Luo², Shawn R. Carlson², Gary R. Rudgers², Jennifer M. Mach², Eric Grunden², Pierluigi Barone² and Daphne Preuss²

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For humans to efficiently use micro-gravity environments will require a wide array of engineering efforts including mechanical, environmental and biological engineering. Sustainable food, fiber, energy and even pharmaceutical sources will rely entirely or in part on plant sources.

However, existing crops have been selected for terrestrial gravity environments. Adaptation to micro-gravity environments will likely require alteration of multiple genes. Engineered mini-chromosomes (MCs), constructed using a bottom-up approach, allow simultaneous delivery of multiple genes to plants. We have constructed DNA vectors carrying centromere sequences, delivered purified constructs to embryogenic tissue, and identified recombinant molecules that form autonomous MCs. These vectors have been propagated in several genetic backgrounds with reporter genes expressed through multiple generations.

Analysis of inheritance patterns demonstrates that MCs can be transmitted through somatic or sexual cell divisions; overall frequencies of inheritance approach Mendelian expectations. Moreover, visual assays (fluorescent *in situ* hybridization or FISH) show co-localization of marker genes, centromere sequences and DNA-staining bodies that are much smaller than the host plant chromosomes. This novel approach for plant transformation and engineering may provide the answer to challenges such as microgravity environments that require multi-gene approaches.

Special Lecture

**What is Required to Maintain
Skeletal Muscle Health in
Space?**

[25]

WHAT IS REQUIRED TO MAINTAIN SKELETAL MUSCLE HEALTH IN SPACE?

D.A. Riley and J.M. Van Dyke. Depart of Cell Biol, Neurobiol & Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226.

Skeletal muscle is an amazingly responsive tissue that adapts throughout our lifetime to optimize its function to satisfy current working demands. During spaceflight, adaptation is appropriate for movements in reduced gravity, but the memory of the demands in Earth's gravity rapidly fades. The result is inappropriate adaptation for function in a gravity-loaded environment. Daily exercise did not prevent calf muscle atrophy during six-month campaigns aboard the International Space Station (2002-2005). An exercise prescription for preserving muscle health requires maintaining 1) force output, 2) structural strength, 3) range of motion, and 4) endurance. Preservation of contractile and structural strength is targeted by body loading with bungee cords tethered to the treadmill. Maintaining fatigue resistance is addressed with bicycle ergometry. Inadequate attention has been paid to range of motion. In space, the feet assume a plantarflexed posture which puts the calf muscles in a shortened state for long periods. Adaptation to this shortened state is undesirable because the calf muscle range of motion decreases. The shorter muscle length is suboptimal for force generation during weightbearing activity on Earth. Rat hindlimb suspension unloading induces plantarflexion and shortening adaptation of the soleus. Twenty min/day of passive static stretch counters this adaptation and the associated accelerated loss of contractile proteins. Achilles tenotomy in the rat, to simulate tendon rupture, generates an even more pronounced, chronic state of shortening. The severity of contractile protein loss is greater, but 20 min/day of passive static stretch significantly counters this deterioration. The exercise venues utilized on the space station lack sufficient stretch stimulation to prevent muscle adaptation to the plantarflexed posture. An effective exercise prescription must incorporate sufficient stretch magnitude and duration to preserve range of motion. A successful prescription for maintaining muscle health during long-term spaceflight will also benefit individuals on Earth requiring prolonged clinical bed rest.

Oral Session I

**Gravitational and Space
Biology: Plants**

Chair - Chris Brown

[26]

THE GRAVITY PERSISTENCE SIGNAL MUTANTS OF ARABIDOPSIS THALIANA: INSIGHTS INTO GRAVITROPIC SIGNAL TRANSDUCTION. S.E. Wyatt¹, B. Justus¹, N. George¹, C. Schenck¹, C. Bruggeman¹, K. Shen¹ and D. Luesse². ¹Dept. of Environmental & Plant Biology, Ohio University, Athens, OH and ²Dept. of Biological Sciences, Southern Illinois University, Edwardsville, IL.

The gravitropic response of *Arabidopsis* inflorescence stems is rapid with a visible response within 30 min and vertical reorientation within 2 h. However, horizontal gravistimulation at 4°C does not cause curvature. When the stems are subsequently returned to vertical at RT, they bend in response to the previous, horizontal gravistimulation. These results indicated that gravity perception can occur at 4°C, but that part of the response is sensitive to cold. At 4°C, amyloplasts in the endodermis of the inflorescence stems sediment normally, but auxin transport was abolished indicating that the cold treatment affected early events of the signal transduction prior to auxin transport. The *gravity persistence signal* (*gps*) mutants of *Arabidopsis* were selected from a T-DNA tagged population using this cold effect on gravitropic signal transduction. To date, the screen has produced seven unique mutants: *gps1*, *gps4* and *gps7* show no response to the cold gravistimulation, *gps2* and *gps6* bend the wrong way, and *gps3* and *gps5* both over-respond to the cold gravistimulation. The defects caused by the mutations affect auxin redistribution, as indicated by expression of an auxin responsive promoter::GUS fusion, as might be predicted by the phenotype, further indicating that the defects are upstream of auxin. Elucidation of the role of the genes defective in these mutants may provide insight into the molecular mechanisms of gravity signal transduction.

(Partially supported by NSF: 0618506)

[28]

GRAVITY-MODULATED TRANSPORTERS: MOLECULAR CANDIDATES THAT COULD DRIVE THE TRANS-CELL CALCIUM CURRENT IN SINGLE-CELLED SPORES OF CERATOPTERIS RICHARDII. S. J. Roux¹, T. Bushart¹, G. Clark¹ and D. Marshall Porterfield². ¹Molecular Cell & Developmental Biol., Univ. of Texas, Austin, TX, ²Dept. Agricultural & Biological Engineering, Dept. of Horticulture & Landscape Architecture, Bindley Biosci. Center-Physiological Sensing Facility, Purdue Univ., West Lafayette, IN.

During the first 18 h after single-celled spores of the fern *Ceratopteris richardii* are induced to germinate by light, gravity fixes the polarity of the subsequent downward nuclear migration and downward rhizoid emergence in these cells. Recent data using calcium-selective electrodes and silicon microfabricated sensor arrays indicate that gravity can induce very rapid changes in the direction and magnitude of a trans-cell Ca²⁺ current in these cells before nuclear migration begins at ca. 24 h into the germination process. Searches in an EST library of genes expressed 20 h after the spores are induced to germinate by light revealed a gene encoding a plasma membrane-type Ca²⁺ATPase, and subsequent quantitative RT-PCR studies showed that this gene is strongly expressed during the peak of the gravity-directed Ca²⁺ current, about 10 h after germination induction. Plant annexin genes can function as Ca²⁺ channels *in vitro*, and two annexin genes are also expressed during the period in which gravity fixes the polarity of the cells. Confirmation of these initial results will be tested and extended by 454 deep sequencing, which will also identify other key genes being expressed 10 h after germination induction. Chemical inhibitors of channel activity or of pump activity both suppress the Ca²⁺ current, but only channel blockers inhibit the ability of gravity to direct cell polarity. These results imply that the force of gravity can rapidly modulate the transport activity of both pumps and channels, but its effects on channels play a more important role in controlling the subsequent polarization events. Molecular and technical insights gained from these studies could be applied to test whether channels and pumps are key participants in the gravity responses of other cells. Supported by NASA grants NAG2-1586 and NAG10-295 to SJR and NNX09AH45G to DMP.

[27]

BASIC CHARACTERIZATION OF THE GRAVITY PERSISTENT SIGNAL 5 MUTANT IN ARABIDOPSIS THALIANA. D.R. Luesse¹, H.T. Huynh¹, J. Kinsler¹, and S.E. Wyatt². ¹Department of Biological Sciences, Southern Illinois University Edwardsville. ²Department of Environmental and Plant Biology, Ohio University.

A plant's ability to tailor its development based on environmental factors is critical for survival. This is accomplished through numerous signals that are regulated by transcription, translation, protein modification, and degradation. In plants, controlled protein degradation plays a critical role in the function of most major growth and development pathways. The specificity of this process is likely mediated by the more than 1300 E3 ubiquitin ligases, which are responsible for facilitating the transfer of ubiquitin to the target. The *gravity persistent signal 5* (*gps5*) mutant, an E3 ubiquitin ligase, was originally identified due to hyper-gravitropism in the inflorescence stem after a cold treatment. The purpose of this study was to perform basic characterization of the *gps5* mutant. Subsequent experiments indicated that the hyper-gravitropic phenotype was also manifested in roots and etiolated hypocotyls. These tissues showed increased growth rates, possibly explaining the hyper-gravitropism. Under light-grown conditions on vertical plates, *gps5* roots grew an average of 190mm, while wild type grew just 75mm. Infrared images of etiolated roots and hypocotyls show enhanced growth under these conditions as well. In addition, *gps5* roots on vertical plates displayed a right-handed curvature of approximately 20°, beginning about four days post germination.

[29]

FUNCTIONAL CHARACTERIZATION OF A GRAVITY-REGULATED STEROL-BINDING PROTEIN IN ARABIDOPSIS THALIANA ROOTS. J.D. Kajla, C.S. Brown, H.W. Sederoff. Dept. Plant Biology, North Carolina State University, Raleigh.

Plants use light and gravity to orient their direction of growth. Roots grow towards the vector of gravity to anchor the plant in the soil and find water and nutrients. Changes in environmental conditions cause changes in gene expression that affect the plants' response. We identified gravity-induced fast and transiently up-regulated transcripts in *Arabidopsis* root apices (Kimbrough et al. 2004). One of these transcripts, for gene At2g16005, is a root specific MD2 lipid binding domain containing protein. Further characterization of At2g16005 showed that it is also regulated by in response to directional light with the same kinetics as its response to gravity (Salinas-Mondragon et al. 2005). Computational analysis showed the protein At2g16005, has a high structural similarity to protein NPC2, malfunctioning of which causes the lethal Niemann Pick disease in human infants. The recombinant protein At2g16005 binds with to the plant sterols stigmasterol and sitosterol, and with a lower affinity to cholesterol. Immunolocalization shows that At2g16005 is localized in vesicular membranes at the root tip. Enhancer trap mutants for the gene show a distinct growth phenotype. About half of the homozygous mutant seeds fail to germinate and grown mutant plants are smaller in size than the wild type plants. Preliminary data show that while the mutant roots bend faster in response to gravity stimulation, the shoots show a delayed bending response. Further experiments to understand the role of this protein in plants, and its associations with other plant proteins are underway. (This work is supported by NASA).

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[30]

DIFFERENTIAL REGULATION OF PLANT MIRNAS IN RESPONSE TO GRAVITY

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Plants harbor a complex population of small RNA (sRNA), including microRNAs (miRNAs), that play critical roles in various developmental programs and stress responses. The function of miRNAs in gravitropism has not been studied. We generated sRNA profiles of *Arabidopsis* root apices derived from gravity stimulated (30 min reorientation) and control plants using high-throughput sequencing (454 Genome Sequencer™ FLX System). We used wild-type (wt) and RNA-DEPENDENT RNA POLYMERASE2 (*rdr2*) knockout mutant plants that have been shown to be defective in siRNA biogenesis. We obtained about 650,000 total sequence reads from 8 different sRNA libraries comprising both gravity-stimulated and control samples. Out of 317,834 non-tRNA/rRNA reads matching the nuclear genome, 43,342 were unique non-redundant sequences. Our combined sRNA sequence database contains miRNA sequences comprising 44 families. Mir160, Mir166, Mir156, Mir846 and Mir169 were among the most abundantly sequenced miRNAs derived from the root apices. We used Real-time qPCR to compliment the sequencing data. The qPCR data confirmed the sequencing results, i.e. the most frequently sequenced miRNAs were found in high abundance using qPCR. With qPCR, we were also able to detect many other miRNAs that were not seen using 454 sequencing. We then compared the miRNA profiles of gravity stimulated root apices and vertical control tissue and found 73 miRNAs that were differentially abundant. In total, 61 miRNAs were significantly more abundant after 30 min of gravity stimulation, whereas 12 miRNAs were down-regulated after gravity stimulation. For example, upon gravity stimulation approximately 4-fold increases in miRNA abundance were observed for miR841 and miR414, while a 2-fold reduction in miRNA161.2 and miR777 abundance was observed. The targets and function of these differentially gravity regulated miRNAs are unknown. We are currently validating additional putative novel miRNAs. (This research was funded by NASA NNX08AR20G.)

[31]

LESSONS LEARNED FROM TROPI-1, A SPACEFLIGHT EXPERIMENT TO STUDY PLANT TROPISMS.

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Phototropism and gravitropism are two physiological processes, and the interactions between these two tropisms determine the final growth form of plants. Although gravitropism can easily be studied on earth, it is difficult to determine the response of roots to light. Therefore, we have developed a project (TROPI, for tropisms) to determine the phototropic response of *Arabidopsis* plants in the microgravity conditions of space. European Space Agency (ESA) has developed a research facility termed European Modular Cultivation System (EMCS) for growing plants in microgravity. The EMCS provides an incubator with two centrifuges and provides atmospheric control, lighting, and high-resolution video. Working with NASA, we have developed experimental unique equipment (EUE) for growing *Arabidopsis* seedlings during spaceflight. The EUE consists of five seedling cassettes with LED lighting and a water delivery system. We have performed several biocompatibility tests in order to optimize the growth of *Arabidopsis*. Our initial studies showed poor seed germination and seedling growth during long-term storage in the EUE. We determined that the conformal coating of the electrical components of the EUE resulted in release of toxic gases inhibiting the growth of seedlings. We added activated carbon filters to the seedling cassettes and to the base of the EUE, and this improved growth of seedlings. We have also initiated additional biocompatibility tests in the space hardware to determine the cause of poor seed germination in some samples. A follow-up experiment has been planned to be performed at intermediate gravity accelerations similar to levels found on the Moon and Mars. (Supported by NASA Grant NCC2-1200).

[32]

THE INTERACTING EFFECTS OF CO₂ AND HYPOBARIA ON GROWTH AND TRANSPIRATION OF RADISH.

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Dept. of Agricultural & Biological Engineering, Uni. of Florida, Gainesville.

Plants will be grown at reduced pressures (i.e., hypobaria) during long-term space missions. In hypobaria, plants can have increased transpiration rates compared to plants that are grown at higher pressures. To study the interacting effects of pressure and partial pressure of CO₂ (pCO₂) we studied the growth of radish plants at different pCO₂ (40 Pa, 100 Pa and 180 Pa) and different total atmospheric pressures (33, 66 or 101 kPa) for seven days. In general, the plant growth and transpiration rates were enhanced with CO₂ enrichment and with decreased total pressure. In limited pCO₂ (40 Pa), the transpiration for plants grown at 33 kPa was over twice that of controls (101 kPa total pressure with 40 Pa pCO₂). Increasing the pCO₂ from 40 Pa to either 100 or 180 Pa reduced the transpirations at all pressures. The leaves of plants grown at lower total pressures (33 and 66 kPa) and super-elevated pCO₂ (180 Pa) had pigment chlorosis. Since water will be a limiting factor for space grown plants, we studied the water use efficiency (WUE) of plants grown at different pCO₂ levels and total pressures as those described above. Increasing pCO₂ increased the WUE while reduced total pressure decreased the WUE. The measured WUE correlated with the predicted WUE values for all pressure and pCO₂ treatments except for plants grown at super-elevated pCO₂ (180 Pa) with lower total pressures. This suggests that very high levels of pCO₂ may result in damages to the mechanisms that promote WUE in plants. Overall, increasing the pCO₂ up to 100 Pa increases the WUE of plants grown in hypobaria.

(Supported by FL Space Grant Consortium: SPACE INIT 2004-2005).

[33]

THE FLAVONOL QUERCETIN REGULATES ROOT GRAVITROPISM AND AUXIN TRANSPORT AND SHOWS AUXIN INDUCED SYNTHESIS

D.R. Lewis¹, D. Cooper¹, K.L. Cooper¹ and G.K. Muday¹ Dept. of Biology, Wake Forest University, Winston-Salem, NC.

A critical component of the root gravitropic response is the redistribution of auxin to the lower side of the root elongation zone. Flavonols negatively regulate root auxin transport, are synthesized at the root tip after gravistimulation, and mutants that lack flavonols have a reduced gravitropic response. We asked which flavonols regulate root gravitropism by using a high-resolution morphometric analysis. The *tt4* mutant, which makes neither quercetin (Q) nor kaempferol (K), was compared to wild type and the *tt7* mutant, which makes only K. Both *tt4* and *tt7* exhibit parallel reductions in root gravitropism and stimulations of basipetal transport, consistent with Q acting as the molecule that regulates these processes. We examined the hormonal signals that regulate the synthesis of K and Q by examining gene expression and flavonol accumulation after IAA and ACC treatment. Both compounds increase expression of genes encoding pathway enzymes and induce flavonoid accumulation in the elongation zone, where gravitropic growth occurs. ACC, an ethylene precursor that inhibits root gravitropism, causes K accumulation, but little Q accumulation, while IAA, which positively regulates root gravitropism, induces both molecules. The inhibition of the gravitropic response by ACC is reduced in the *tt4* and *tt7* mutants, suggesting that the ACC inhibition of gravitropism is Q dependent. We are testing the effect of flavonol application on the gravitropic response in Col, *tt4*, and *tt7* to determine if exogenous flavonoids alter the gravitropic response and lateral auxin gradient formation in reoriented roots of these three genotypes. This work identifies a specific role for Q in regulation of basipetal IAA transport and gravitropism and provides insight into the mechanisms that regulate flavonol synthesis. (Supported by NSF Grant # 0820717 to GKM.)

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Oral Session II

Biological Technologies and New Capabilities for Research and Spaceflight

Chair - Adarsh Deepak

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[34]

DEVELOPING A PORTABLE DEVICE FOR ON-ORBIT BLOOD COUNTING. P. Todd¹, J. F. Leary^{2,5}, M. G. Grafton³, R. Byrnes¹, N. A. Thomas¹, and L. M. Reece^{2,4,5}. ¹Techshot, Inc., Greenville, IN, ²Dept. of Basic Medical Sciences, ³Weldon School of Biomedical Engineering, ⁴Bindley Bioscience Center, ⁵Birck Nanotechnology Center, Purdue University, West Lafayette, IN.

Space Station equipment, according to early planning (ca. 1980's), was to include a flow cytometer. At that time most optically activated flow cytometers utilized large gas lasers. Today, in 2009, there is not yet a flow cytometer on the International Space Station (ISS). During this interval a variety of designs, concepts and sponsorships rose and fell, most subjected to rejection for various reasons. Techshot, Inc. in collaboration with Purdue University responded to yet another call for an on-orbit blood cell counter with a design that is executable on the basis of several breakthrough developments. These developments have occurred in microfluidics, optoelectronics, magnetic nanotechnology and digital technology. In the case of microfluidics a valveless design is implemented allowing one-way, single-use fluidic components with flow and optical properties suitable for flow cytometry. Novel optoelectronics have facilitated a fully semiconductor based multistation system of illumination and photodetection sensitive to the fluorescent output of single cells labeled with antibody-conjugated quantum dots. Magnetic nanotechnology has led to the magnetic manipulation of single magnetically labeled cells by shaped magnetic fields. Finally, evolved digital technology allows "three-button" operation of controls, timelines, data collection, wireless communication and display occupying negligible space and power. Each of these requisite subsystems has been demonstrated to perform as would be required in a gravity-independent, battery-operated, hand-held flow cytometer capable of counting total red blood cells, white blood cells and at least three subsets of leucocytes. (Supported by NASA: NNX09CE43P).

[36]

A NOVEL PHARMASAT COMPATIBLE LAB-ON-A-CHIP PLATFORM FOR STUDYING CYANOBACTERIAL GRAVITATIONAL PHYSIOLOGY. A. Ul Haque¹, J.C. Claussen¹ and D.M. Porterfield^{1,2,3}

¹Dept. of Ag. and Biological Eng., ²Horticulture and Landscape Architecture and ³Weldon School of Biomedical Eng.

Cyanobacteria were among the earliest known organisms to inhabit the earth, with fossil records dating back to 3.5 – 3.6 Ga. They played a vital role in development of the global atmosphere via O₂ evolution and carbon fixation. Because cyanobacteria are important in search for life missions it is necessary to understand *cyanobacterial* physiology, especially in non-earth normal gravity. As part of this effort we are developing a novel bioMEMS sensor array selective for bicarbonate, H⁺, and O₂ (CHO biochip) capable of non-invasively measuring these key parameters of *cyanobacterial* photosynthetic activity. Each biochip consists of 14 Pt electrodes fabricated on a SiO₂ insulated Si substrate. Three electrode pairs are for amperometric O₂ measurement with reference electrodes. For potentiometric H⁺ and HCO₃⁻ sensors there are 3 electrodes, with the remaining two electrodes serving as the reference electrodes for these. All the potentiometric electrodes and the O₂ reference electrodes are electroplated with Ag/AgCl. Each electrode is coated with a specific material, based on chemistry to impart selectivity towards O₂, H⁺ and HCO₃⁻ respectively. The final prototype biochip is 10.8 x 11 mm in dimensions. The CHO biochips will be interfaced with a CDBioLab currently in development at NASA AMES. There will be 16 biochips that will interface with 16 wells each on the CDBioLab, with each well forming a *cyanobacterial* culture and measurement chamber. Combined with a centrifuge, the system will ultimately be flown on a PharmaSat analogue nanocube satellite. Variations in *cyanobacterial* extracellular O₂, H⁺ and HCO₃⁻ concentrations in response to gravity fluctuations ranging between µg, Earth, Lunar and Mars g will be measured and characterized. This information is expected to increase our understanding of the role of gravity in microbial cell physiology, and also provide optimum space culture conditions for using *cyanobacteria* as a component of a future Advanced Life Support system. (Supported by NASA)

[35]

SPACEFLIGHT HARDWARE USED TO DIFFERENTIATE BONE MARROW STEM CELLS. P.J. Duke¹, H. Luong¹, W. LeBouef², Q. Diep³, S. Cai⁴. ¹Dept. of Orthodontics, Dental Branch, ²Our Lady of the Lake College, Baton Rouge, LA, ³University of Houston, Houston TX, ⁴Dept. of Endodontics, Dental Branch, Houston TX.

Bone-forming cartilage grown in the rotating bioreactor healed defects in the skulls of mice, but the semi-spherical nodules were not a good fit for the defects. Therefore, we explored other methods of producing a flat piece of cartilage, better suited for implanting in the skull. In the current experiment, spaceflight hardware from our IML1 flight (1992) was used to differentiate bone marrow mesenchymal stem cells into neurons or chondrocytes. Bone marrow cells were isolated and expanded in culture. After 2 passages, cells suspended in medium were inoculated into an upside down BEX (bubble exchange) hardware unit, allowing cells to aggregate on the Silastic membrane. Upside down units in Petri dishes were placed in a 37°C incubator, with or without 5% CO₂ for two hours; then all were placed in CO₂ overnight. After addition of medium and removal of air bubbles, cells were cultured for 3-7 days prior to being fixed, with ½ the medium changed every other day. Results: Cells exposed to no CO₂ during the first two hours of incubation aggregated into small aggregates and occasional large (0.5mm) ones. These cells appeared to be chondrocytes, and stained with alcian blue at low pH. Cells receiving initial CO₂ spread onto the membrane surface, and after 24 hours, they had numerous extended processes and the appearance of neurons. This was confirmed by methylene blue staining for Nissl bodies present in the cytoplasm of neuronal cells. The unique gas environment inside the Silastic bubble caused the cells to go down one or the other pathway, but the desired shape was not realized. Supported by UTHSC Office of Biotechnology.

[37]

REAL TIME PHYSIOLOGY OF SILICA-ENTRAPPED BIOFILMS USED FOR WATER REUSE IN LIFE SUPPORT. E.S. McLamore^{1,2,3}, D. Jaroch^{1,4}, J.L. Rickus^{1,3,4}, D.M. Porterfield^{1,3,4,5}, and M.K. Banks².

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Long duration space exploration and habitation are dependent on efficient water supplies, as water comprises a large fraction of daily crew mass input. Wastewater recovery reduces the need for shuttle resupply, although physico-chemical wastewater recovery technologies are often dependent on consumables. Inclusion of biotreatment technologies reduces this dependence on resupply, and efficient biotechnologies must have low energy, mass, and crew maintenance requirements. One of the biotechnologies currently under consideration for use within life support systems is the membrane-aerated bioreactor (MABR). MABR provide bubble-free gas transfer from the lumen to shell side, where sessile bacteria degrade wastewater. Although much has been learned about MABR using bulk liquid analysis and invasive probing techniques, little is known about the dynamic conditions within the mass boundary layer (MBL) formed at the biofilm-wastewater interface. A novel sensing modality (known as self-referencing) was used in combination with microscopy techniques and bulk liquid analysis for characterizing real time biophysical transport in biofilms within MABR. Following determination of physiological conditions, an advanced cell immobilization technique (porous silica cell immobilization) for reducing detachment of membrane-bound biofilms was investigated. Porous silica immobilization of cells (biosilification) is a biocompatible, optically transparent encapsulation method used for high quality thin film deposition. Results indicate that encapsulated membrane-bound cells within biofilms are viable, retain their morphology, are metabolically active, and are physically trapped following biosilification. Use of this advanced cell immobilization technique will increase the efficiency of MABR within life support systems by reducing uncontrolled biofilm erosion. Ongoing work entails long term analysis of effects of biosilification on membrane-bound biofilm physiology.

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CONDUCTING GRAVITATIONAL BIOLOGY RESEARCH ON SUBORBITAL COMMERCIAL FLIGHTS. Y.D. Cagle¹, E.B. Wagner²
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While commercial suborbital flights are often touted for their ability to support space tourism, they also provide a fresh opportunity for gravitational biology investigations. With longer periods of microgravity than parabolic flight, a more controlled environment than most sounding rockets, and opportunities for crew-tended research, such flights will readily support autonomous payloads, interactive payloads, and bioastronautics investigations with human subjects.

Unmanned suborbital payloads are anticipated as early as 2010, with crewed flights as soon as 2011. With market predictions in the hundreds of flights per year, one can readily envision a robust flight research program with frequent and repeated access to short microgravity exposures (3-5 minutes). We describe the breadth of flight environments and accommodations in development by the major flight providers, highlight subspecialties of gravitational biology particularly well served by these experimental conditions, and suggest novel opportunities not supported on other existing flight platforms.

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A NOVEL BEDREST ANALOG OF LUNAR EXPLORATION. A.M. Hanson¹, A.J. Rice¹, S. Novotny¹, K.O. Genc¹, M. Kuklis², A. Licata², P.R. Cavanagh¹, ¹Dept. of Orthopaedics & Sports Medicine, Univ. of Washington, Seattle, WA, ²Cleveland Clinic Center for Space Medicine, Cleveland, OH.

Bedrest is an excellent analog for the study of musculoskeletal changes that occur in the microgravity of long-duration spaceflight. However, existing bedrest models are unsatisfactory simulations of partial gravity environments due to the complete removal of lower extremity loads. Thus there is no basis at present for determining if working in lunar (1/6g) or Martian (1/3g) settings will be osteoprotective. We performed a feasibility study of a bedrest model in which 5 subjects experienced 1/6g loads on the plantar aspect of the feet over the course of a 6-day study. Subjects were allowed to stand, sit, and perform typical daily activities and other weight bearing activities while in the 1/6g configuration. Parameters examined include perceived comfort, foot forces, urine Ca⁺, and plasma volume. Initial discomfort was reported primarily in the lower back, ankles, and feet, but, by day 6, values were in the tolerable range. Foot force measurement showed that average loading of ~0.16g was achieved. Urine Ca⁺ excretion was elevated over the six day study, and plasma volume decreased on average by -7.9%. While limitations to the model exist, this study demonstrates the feasibility of using a loaded 9.5 degree head-up tilt bedrest model as an analog for examining physiological adaptations during lunar missions and that longer duration studies are now required.

Acknowledgments: We are grateful to Dr. Steve Platts for performing the plasma volume measurements and to Dr. Bill Paloski for his collaboration.[supported by NASA: NNC08QA14P.]

[39]

MAGNETIC LEVITATION OF HUMAN A431 CELLS M.J.A. Moes¹, J.C. Gielen², R. Bleichrodt¹, J.J.W.A. van Loon^{3*}, P.C.M. Christianen², J. Boonstra¹ - ¹Institute: Cellular Architecture and Dynamics, Utrecht University, NL. www.bio.uu.nl. ²High Field Magnet Laboratory (HFML), Radboud University Nijmegen, NL. www.hfml.ru.nl/peterc. ³Dutch Experiment Support Center (DESC) @ Dept. Oral Cell Biology, ACTA, Vrije Universiteit, Amsterdam, NL. www.descsite.nl.

During the last decades a wide variety of experiments during space flights have demonstrated that gravity has profound effects on whole organisms, organs and tissues. Interestingly, the virtual absence of gravity also had profound effects on the cellular and molecular level, including changes in cell morphology, modification of gene expression, changes in signal transduction cascades and even changes in the self-organization of tubulin. One of the gravity sensitive components in cells appears to be actin. Our experiments in real microgravity using sounding rockets revealed a modified actin cytoskeleton of A431 epidermoid carcinoma cells in space resulting in the rounding of these cells and an increased polymerization of actin. Actin is a major component of the cytoskeleton and has important functions, amongst other in signal transduction, motility, attachment, and cell morphology.

The aim of the present research was to use magnetic fields as analogues for real microgravity to study the effect of levitation on the actin cytoskeleton in human A431 cells, in order to establish the potential of magnetic levitation as a simulation of microgravity conditions. We compare the results with data found in the past in real microgravity and in simulated microgravity using the fast rotating clinostat and RPM. During magnetic levitation cells are exposed to high magnetic fields. Therefore we studied also the effect of such a magnetic field on the cells without levitation. Human A431 cells were exposed to magnetic levitation for different time intervals and chemically fixed while levitation was ongoing. Subsequently the actin morphology and behaviour of focal adhesions were investigated using fluorescence microscopy. The behaviour of focal adhesions is an indicator for attachment and rounding or spreading of cells. Identical results were obtained in the RPM studies and magnetic levitation studies. However, controls for the effect of the magnetic field raised concern about the potential of magnet simulated microgravity and indicated the importance of this control.

This study was supported by SRON-NWO grant MG-059 and MG-057 and part of this work has been supported by EuroMagNET under EU contract RII3-CT-2004-506239.

[41]

JAPANESE RESEARCH GROUP FOR ANIMAL AND HUMAN PHYSIOLOGY IN LUNAR AND MARTIAN GRAVITY. Y. Kumei¹, J.L. Zeredo², T. Yabushita¹, T. Ishida¹, S. Seki¹, T. Ikeda³, K. Toda³, M. Matsuura⁴, Y. Nomura⁵, K. Iwasaki⁵, F. Kawano⁶, Y. Ohira⁶, M. Okuno⁷, D. Kageyama⁸, and M. Yamashita⁹. ¹Tokyo Medical and Dental University, Tokyo 113-8549, ²University of Brasilia, Brasilia 71620-275, Brazil, ³Nagasaki University, Nagasaki 852-8588, ⁴Japanese Foundation for Cancer Research, Tokyo 135-8550, ⁵Nihon University, Tokyo 173-1861, ⁶Osaka University, Osaka 560-0043, ⁷University of Tokyo, Tokyo 153-8902, ⁸Diamond Air Service Inc., Aichi 480-0293, and ⁹JAXA/ISAS, Kanagawa 229-8510, Japan.

We have organized the first and only research group in Japan dedicated to promoting life sciences researches in Lunar and Martian gravity. For this primary purpose, our flight experiments are conducted under the partial gravity conditions that are generated by original parabola trajectory aboard Mitsubishi aircraft MU-300 or Gulf Stream-II of Diamond Air Service Inc. Our flight experimental system provides a wide selection of gravity levels precisely in cooperation with JAXA supporting system. Effects of the partial gravity are examined at the levels of organism/whole animal, tissue, cell, and molecule. We have successfully completed flight campaigns for studies on central nervous system, vestibular function, muscle-nerve reflex, and male reproductive cell function. On-going projects include sensory-motor system, bone metabolism, and cardiopulmonary function. Our research group also looks out to establish collaborative works and increase the availability of our unique parabolic flights to other research groups both in Japan and abroad. (Supported by JAXA for FY2007, 2008, and 2009)

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ADVENTURES OF THE AGRONAUTS. J.B. Cook, C.S. Brown, NC Space Grant, NC State University, Raleigh, NC.

Adventures of the Astronauts is a free, online science curriculum with a space biology theme for elementary and middle school grade level students. This curriculum was created to meet the NC Standard Course of Study for both 3rd and 6th grade Science. There are separate “missions”, each of which includes writing activities and inquiry-based experiments. The missions can be used as a complete set or individually. The online format allows for different presentations of the curriculum: students can go to a computer lab and read the material individually or as a group; teachers can project the curriculum onto a screen; or the curriculum can be printed. In addition to the online modules, supplemental activities have been developed in partnership with BioServe Space Technologies in Boulder, CO that bring real-time research into classrooms via the internet (ISS Expeditions 14 and 15). Students have the opportunity to participate in ground experiments to compare with flight experiments. Teachers have access to inquiry-based lessons that correlate with both ground and flight experiments.

(Supported by NASA and NC Space Grant)

[44]

GENE EXPRESSION CHANGES IN SPACE FLOWN CAENORHABDITIS ELEGANS EXPOSED TO A LONG PERIOD OF MICROGRAVITY. R. Jamal¹, J. Nurul-Faizah¹, S.M. Then¹, S. Nathan², N.J. Szewczyk³, L.S. Stodieck⁴, R. Harun¹. ¹UKM Medical Molecular Biology Institute (UMBI), Universiti Kebangsaan Malaysia, ²Malaysia Genome Institute, ³University of Pittsburgh, USA, ⁴Bioserve, Colorado, USA.

Scientists are trying to determine the long term effects of microgravity and space radiation on various cellular and biological functions. We used the *Caenorhabditis elegans* as the model organism to study the changes in gene expression. Wild type worms (Univ. of Pittsburgh) were grown in the liquid medium CeMM using the CHab hardware (Bioserve), which consisted of 6 interconnecting opticells. The CHab was flown as part of the CGBA Science Insert-01 (CSI-01) on the STS116 flight (December 2006) to the International Space Station. The worms were incubated in the CGBA and were passaged every month from one opticell to the next. The CHab returned to earth on the STS118 flight in July 2007. Upon landing, surviving worms were extracted and RNA later added. Ground controls were grown at Bioserve and passaged accordingly. RNA was later extracted and mRNA gene expression was analysed using Affymetrix GeneChip® *C. elegans* Genome Array. The results revealed that out of 22 625 genes, 1228 genes were differentially expressed ($p \leq 0.05$ and fold changes $\geq \pm 2$): 906 genes were up-regulated and 322 genes down-regulated. From this list, *dod-19*, *dod-6* and *dod-3*, the downstream effectors of the forkhead transcription factor *daf-16*, were up-regulated. *Daf-16* regulates insulin/TGF signaling pathway that influence metabolic alterations, increased stress and microbial resistance. The glutathione S-transferase (*gst-13*, *gst-17*, *gst-26*, *gst-27*, *gst-36*) and radiation sensitive gene (*rad-51*) were up-regulated suggesting responses to high oxidative stress. The down-regulation of muscle growth genes (*myo-3*) maybe due to reduced mechanical stress in muscle exposed to long term microgravity.

Conclusion: Long term exposure of *C. elegans* to microgravity suggests changes in genes involved in ageing, oxidative stress and muscle growth. (Supported by UKM-ANGKASA-NBD-0019-2007)

[43]

EFFECTS OF ACTIVE HEAT SHOCK FACTOR 1 ON SKELETAL MUSCLE HYPERTROPHY IN MICE. K. Goto¹, Y. Ohno¹, A. Nakai², T. Sugiura³, Y. Ohira⁴ and T. Yoshioka⁵. ¹Lab. of Physiol., Sch. of Health Sci., Toyohashi SOZO Univ., Toyohashi; Depts. of ²Biochem. Mol. Biol., Grad. Sch. Of Med. and ³Exerc. Health Sci., Yamaguchi Univ., Yamaguchi; ⁴Grad. Sch. of Med., Osaka Univ., Toyonaka; ⁵Hirosaki Gakuin Univ., Hirosaki, Japan.

Mechanical stress as well as heat stress activate proliferative potential and induce muscle hypertrophy. On the other hand, it has been suggested that up-regulation of heat shock proteins (HSPs) induced by heat stress may be one of the signals for muscle hypertrophy. Although it has been proposed that HSPs has several cellular functions including the protection of cells from various extracellular stressors, the precise mechanism, as well as the physiological roles, for HSPs induction in skeletal muscles is still not fully understood. The induction of HSPs expression is regulated by heat shock factor 1 (HSF1), which binds to heat shock elements located on the upstream region of all *Hsp* genes. In the present study, using the transgenic mice expressing the active form of HSF1 (Tg-HSF1), we examined the role of HSPs in the overloading-associated hypertrophy of skeletal muscles. Functional overloading on soleus was induced by tenotomy of synergistic muscles, and was maintained for 4 weeks. Increase in overloading-induced protein content and up-regulations of HSPs of soleus muscles were observed compared with that of wild type animals. Results from this study indicated that the up-regulation of HSPs has strong facilitated effect on the protein synthesis.

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[45]

NOREPINEPHRINE STIMULATES OR INHIBITS CONTRACTION OF FIBROBLAST POPULATED COLLAGEN GELS DEPENDING ON ITS CONCENTRATION. B. P. Johnson-Wint. Dept. of Biological Sciences, Northern Illinois University, DeKalb, IL.

Fibroblast populated collagen gels (FPCGs) contract in vitro. During contraction FPCGs change morphologically from a larger diffuse to a denser compact collagen containing synthetic tissue. Fibroblasts produce the contraction force using cytoplasmic beta-actin filaments and myosin and are sensitive to mechanical load. Since fibroblast adhesion, motility, proinflammatory responses and paracrine signaling are known to be influenced by adrenergic receptor pathways the purpose of the present study was to determine if norepinephrine also influences FPCG contraction. FPCGs were formed from adult rat tail tendon fibroblasts (10^5 cells/100 μ l) in DME (low glucose) containing 5% calf serum and native rat type-I collagen (2.4 mg/ml) fibrils and were exposed to 7 concentrations of norepinephrine (1, 10, 20, 50, 100, 200 and 1000 nM) for 95 hours. FPCGs were photographed at 0.75, 23, 48, 71 and 95 hours and the area of the FPCGs measured at each time to track gel contraction. The norepinephrine dose response curve showed both an inhibitory and stimulatory effect on FPCG contraction. Norepinephrine inhibited FPCG contraction up to 210% at 100 nM while it stimulated FPCG contraction up to 50% at 200 nM. Norepinephrine had no effect on FPCG contraction at 1, 10, 20, 50 and 1000 nM. These results demonstrate a narrow concentration window dual effect of norepinephrine on FPCG contraction. Norepinephrine is stimulatory at 200 nM and inhibitory at 100 nM. This may indicate different mechanisms of action on the fibroblasts at the 2 concentrations and may be of morphological significance to the building of dense connective tissue. (Supported by NIU and the NIU Foundation.)

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SIMULATED MICROGRAVITY: A NOVEL APPROACH TO EMBRYONIC STEM CELL CULTURE. Y. Kawahara^{1,2}, T. Manabe³, M. Matsumoto³, T. Imura³, T. Kajiume¹, M. Takada¹, M. Matsumoto¹, and L. Yuge^{2,3}. ¹Graduate School of Biomedical Sciences, ²Space Bio-Laboratories Y. K., ³Graduate School of Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, JAPAN.

We have reported that simulated microgravity using 3D-clinostat inhibited stem cells differentiation such as human mesenchymal stem cells, mouse hematopoietic stem cells, and mouse bone marrow stromal cells. Embryonic stem (ES) cells readily proliferate and differentiate but also undergo unexpected spontaneous differentiation. Leukemia inhibitory factor (LIF) is an indispensable factor for maintaining mouse ES cell pluripotency. A feeder layer and serum are also needed to maintain an undifferentiated state, however, such animal derived materials need to be eliminated for clinical applications. Therefore, a more reliable ES cell culture technique is required. In this study, we demonstrated mouse ES cells were cultured in normal 1G condition (group 1G) or 10⁻³ G condition (group CL), using feeder-free and serum-free medium of ESF-C medium without LIF. Cells in group 1G showed morphologically differences in normal ES cells. On the other hands, cells in group CL formed many small spheres after 3 days of culture, and these spheres were bigger during culture period. RT-PCR and ALP staining indicated the spheres were undifferentiated mouse ES cells. Moreover, teratomas were generated by subcutaneously injecting group CL cells. Here we show that simulated microgravity allows novel LIF-free and animal derived material-free culture methods for mouse ES cells. Our results suggest that simulated microgravity provides a straightforward and effective means for stem cell culture.

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CHANGES IN GENE EXPRESSION OF HEPG2 CELLS EXPOSED TO MICROGRAVITY. A.A.N. Khairul-Bariah¹, S.M. Then¹, R. Rageshwary¹, N. Fazlina¹, W.N. Wan-Zurinah¹, H. Roslan¹, D.M. Klaus², L.S. Stodieck², R. Jamal¹. ¹UKM Medical Molecular Biology Institute (UMBI), Universiti Kebangsaan Malaysia, ²Bioserve & University of Colorado, USA.

Previous studies have shown that cytoskeletal changes and increased expression of key transcription factors in HepG2 liver cancer cell line grown under simulated microgravity conditions. This study was aimed at determining the effects of microgravity on the gene expression profile of HepG2 cells grown in 3-dimensional culture at T₀ and T₇₂ and also ground samples. A set of 9 Fluid Processing Apparatus (FPAs) were used (Bioserve, USA) for this component of the experiment. The 3 compartments contained initial seeding of 1.5 X 10⁶ HepG2 cells grown on OPLA 3D-scaffold in complete growth medium (CGM), carbonized CGM and the fixative RNAlater® respectively. Triplicates were used for T₀, T₇₂ and ground controls in Baikonur that were fixated at time of launch (10th Oct 2007). Flight samples were flown on Soyuz TMA-11 and transferred shortly after docking into the Kryogem set at 37°C for 24 hrs. Activation was performed on 13th Oct 2007 by the Malaysian space flight participant by adding culture medium into cells. After 30 minutes, a set of 3 FPAs was terminated (designated as T₀) by adding RNAlater®. The other 3 FPAs were allowed to incubate at 37°C for 72 hrs before termination (T₇₂). FPAs were kept at 4°C until packing and return. RNA was extracted and microarray analysis performed using the GeneChip® Human gene 1.0 ST Array. Analysis showed that 116 genes were differentially expressed (73 genes upregulated, 43 genes downregulated) between pre-launch samples compared to samples at T₀. There were 38 genes that were differentially expressed (18 genes upregulated, 20 genes downregulated) between T₀ and T₇₂. Some of these genes are involved in transcription and translation regulation, zinc finger domain, ion channels, cell adhesion, metabolism and membrane structure. **Conclusion:** Key changes in gene expression were observed in HepG2 cells during the 72-hour growth culture period in microgravity as well as in comparison with pre-launch ground samples. (Supported by MOSTI grant UKM-ANGKASA-NBD0018-2007)

[48]

LESSONS LEARNED FROM TROPI-1, A SPACEFLIGHT EXPERIMENT TO STUDY PLANT TROPISMS. P. Kumar, R.E. Edelmann, J.Z. Kiss. Department of Botany, Miami University, Oxford, OH.

Phototropism and gravitropism are two physiological processes, and the interactions between these two tropisms determine the final growth form of plants. Although gravitropism can easily be studied on earth, it is difficult to determine the response of roots to light. Therefore, we have developed a project (TROPI, for tropisms) to determine the phototropic response of *Arabidopsis* plants in the microgravity conditions of space. European Space Agency (ESA) has developed a research facility termed European Modular Cultivation System (EMCS) for growing plants in microgravity. The EMCS provides an incubator with two centrifuges and provides atmospheric control, lighting, and high-resolution video. Working with NASA, we have developed experimental unique equipment (EUE) for growing *Arabidopsis* seedlings during spaceflight. The EUE consists of five seedling cassettes with LED lighting and a water delivery system. We have performed several biocompatibility tests in order to optimize the growth of *Arabidopsis*. Our initial studies showed poor seed germination and seedling growth during long-term storage in the EUE. We determined that the conformal coating of the electrical components of the EUE resulted in release of toxic gases inhibiting the growth of seedlings. We added activated carbon filters to the seedling cassettes and to the base of the EUE, and this improved growth of seedlings. We have also initiated additional biocompatibility tests in the space hardware to determine the cause of poor seed germination in some samples. A follow-up experiment has been planned to be performed at intermediate gravity accelerations similar to levels found on the Moon and Mars. (Supported by NASA Grant NCC2-1200).

[49]

NEW INSIGHTS INTO PHOTOTROPISM FROM EXPERIMENTS IN MICROGRAVITY. K. D. Millar¹, P. Kumar¹, and J.Z. Kiss¹

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Gravitropism and phototropism were studied in a series of microgravity experiments on the International Space Station (ISS). Specifically, the role of red-light-absorbing phytochrome pigments in modulating tropisms in seedlings of *Arabidopsis thaliana* was investigated. The response of the WT was compared to *phyA*, *phyB*, and *phyAB* mutants. These studies were performed on the European Modular Cultivation System (EMCS), which provides an incubator with atmospheric control, lighting, and high resolution video. The EMCS has two rotating centrifuge platforms so that experiments were performed at 1g (as a control) as well as in microgravity (centrifuge off). The main advantage of these space experiments is that a “pure” phototropic response can be studied without the “complications” of a constant gravity vector as found on Earth. Seed germination (57%) during the space experiments was lower than ground controls (>90%), and this was likely due to extended storage of 8 months in flight hardware. However, the seedlings that germinated exhibited robust growth and tropistic curvature. Both roots and hypocotyls from seedlings that developed in microgravity had a stronger phototropic curvature (in response to the various light qualities tested) compared to seedlings in the 1g control. The broader implications of these results for models of phototropism will be discussed in this presentation.

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EFFECT OF HYPERGRAVITY EXPOSURE ON ABUNDANCE OF TRANSCRIPTS ASSOCIATED WITH FREE FATTY ACID TRANSPORT IN RAT MAMMARY GLAND.

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During lactation, the mammary gland is programmed to utilize free fatty acids (FFA) released from other tissues to make milk fat to meet the caloric needs of the neonate. The transfer of FFA in cells is regulated by putative transport proteins including caveolin (CAV) and Fatty acid translocase (FAT). Prolactin (PRL) and glucocorticoids (GC) play key roles in directing mammary metabolism. It is known that in-vitro incorporation of glucose into lipids is greatly inhibited in mammary tissue from pregnant and lactating rats exposed to chronic hypergravity (HG). Therefore the objective of this study was to determine the effects of periparturient HG exposure on transcript levels of CAV1, CAV2 and FAT in the lactating rat mammary gland. Pregnant rats were exposed to either 2g (HG) or kept at 1g (control) from day 11 of gestation (G11) through Postnatal day 1 (P1). HG exposed rats were supplemented with either PRL or GC from G13 to P1. On P1, mammary tissue was collected to measure mRNA abundance of CAV1, CAV2 and FAT by quantitative PCR.

Transcript levels for CAV1 and CAV2 in mammary tissue were *not* affected by exposure to 2g. FAT mRNA abundance was about 60% less in mammary tissue of the HG group compared to control ($p < .001$). Neither PRL nor GC supplementation could override the HG-induced suppression of FAT. Overall, these results indicate that HG has an impact on specific FFA transporters to limit fat synthesis. The hormones, PRL and GC, which are known to be necessary for induction of lactation, were not able to alleviate FAT mRNA levels in HG-exposed animals. (Supported by NASA NNA05CP91A)

[52]

IMPACT OF MICROGRAVITY ON THE EXPRESSION OF MULTI-DRUG RESISTANT PROTEINS IN JURKAT CELLS.

S.M.Then¹, N.Fazlina¹, M.Hafizah¹, A.A.N.Khairul-Bariah¹, H.Noor-Hamidah², A. Maha³, D.M. Klaus⁴, L.S. Stodieck⁴, R.Jamal¹. ¹UKM Medical Molecular Biology Institute, Universiti Kebangsaan Malaysia, ²Dept of Pathology, Faculty of Medicine, UKM, ³Dept of Pathology, Faculty of Medicine and Health Science, Universiti Putra Malaysia, ⁴Bioserve & University of Colorado, USA.

As multi-drug resistance (MDR) proteins are located and mediated at the cell membrane, we postulated that microgravity may modulate changes to the expression of these proteins. We investigated the effects of microgravity on the expression of MDR proteins Pgp, MRP1, MRP3, MRP4, LRP and BCRP, in space-flown Jurkat cells compared to ground controls at two time points, T₀ and T₇₂. A set of 9 Fluid Processing Apparatus (FPAs) were used (Bioserve, USA) for this component of the experiment. The 3 compartments in each FPA were filled with 1.5×10^6 cells suspended in complete growth medium (CGM), carbonized CGM and 8% paraformaldehyde as the fixative. Triplicates of FPA were used for T₀, T₇₂ and ground controls in Baikonur that were fixated at time of launch (10th Oct 2007). Flight samples were flown on Soyuz TMA-11 and transferred shortly after docking into the Kryogem set at 37°C for 24hrs. Activation was performed on 13th Oct 2007 by the Malaysian space flight participant by adding culture medium into cells. After 30 mins, a set of 3 FPAs were terminated (designated as T₀) by adding paraformaldehyde. The other 3 FPAs were allowed to incubate at 37°C for 72 hrs before termination. FPAs were kept at 4°C until packing and return. Analysis in Malaysia showed the number of cells increased from launch (1.5×10^6) to T₀ but later decreased at T₇₂. Flight samples showed a decreased in MRP1 expression at T₇₂ compared to T₀ ($p < 0.01$). We postulate that microgravity reduces the need for ATP-dependent pathways, hence downregulating proteins like MRP1. Expression of Pgp and BCRP were also significantly decreased in flight samples compared to ground ($p < 0.001$). In conclusion, microgravity caused changes in the expression of key MDR proteins which are mediated through the ATP-dependent mechanism within the cell membrane. (Supported by MOSTI grant UKM-ANGKASA-NBD0018-2007)

[51]

THE EFFECTS OF MICROGRAVITY ON THERMOSTABLE T1 LIPASE PROTEIN CRYSTAL.

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The putative thermostable lipase producer represents a novel species for which *Geobacillus zalihae* sp. nov. is proposed, with type strain T1^T. The quest for the characterizations of intrinsically thermostable T1 lipase either physicochemically or structurally is a prominent task. A thermoalkaliphilic T1 lipase gene was overexpressed in pGEX vector in the prokaryotic system intra- and extracellularly. High yield purification was achieved through two steps affinity chromatography with a final specific activity and yield of 958.2 U/mg and 51.5%, respectively. The T1 lipase was extensively characterized, both physicochemically and spectroscopically using Circular Dichroism (CD) and spectrofluorometry. High temperature crystallization of T1 lipase was a new discovery. The atomic details of T1 lipase solved at 1.5 Å unveiled a novel cation- π interaction which was the first report among thermostable lipases. A mutant F16L has revealed F16 was a key residue in this interaction. Space crystallization of both proteins was carried out using high-density protein crystal growth apparatus (HDPCG) utilizing vapor diffusion method and X-ray diffraction data was collected at SPring-8 BL41XU, Japan. Microgravity apparently improved the size and interface of crystals significantly. Microgravity effect on crystallization of T1 lipase was clearly evidence by the finer atomic details at 1.3 Å. Structure elucidation at high temperature crystallization and microgravity effects helps in the understanding of protein structure in general and in the unfolding process in particular.

[53]

LUNAR BIOLOGY REQUIREMENTS DEVELOPMENT.

D. Reiss-Bubenheim¹, N. Rayl¹ and R. Briggs² NASA-ARC¹ and ²Lockheed Martin Mission Services
NASA is in the process of establishing science requirements for the Constellation Lunar Program. Ames Research Center (ARC) is supporting the Optimizing Science and Exploration Working Group (OSEWG), a NASA HQ group, to develop non-human life science goals and objectives for Lunar sortie and long-duration outpost missions. The ARC support team is soliciting input and vetting from the space biology community via the LEAG (Lunar Exploration Advisory Group) roadmap exercise and via a newly established Lunar Biology Focus group sponsored by the NASA Lunar Science Institute (NLSI).

This poster will review the Constellation requirements, activities of the OSEWG and outline how researchers can get involved with lunar biology opportunities and the NLSI biology focus group.

[54]

THE LIFE AND PHYSICAL SCIENCE LABORATORY (LPS) AND ITS FACILITIES AT ESA'S EUROPEAN SPACE RESEARCH AND TECHNOLOGY CENTRE (ESTEC) IN THE NETHERLANDS

Jutta Krause*, Christel Paille*, Alan Dowson*, Lobke Zuijderduijn*,
Stephanie Raffestin*, Nadine Fritz*, Humberto Cunha**,
Pedro Raposo**, Jack J.W.A. van Loon***

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The Life and Physical Science Laboratory (LPS) of ESA at the European Space Research and Technology Centre (ESTEC) in Noordwijk, the Netherlands is accessible to the scientific community since mid 2008. The LPS is equipped with state of the art laboratory equipment to provide support in the fields of life and physical science, life support and related exploration. Currently it is suited for life science/life support work that requires up to bio safety level 2 (BSL 2). Furthermore the LPS laboratory is equipped with special hyper gravity and micro gravity simulation equipment (the 8m diameter centrifuge offers a maximum of 20G), a variety of molecular biology and anaerobic cultivation facilities as well as an insulated higher plant cultivation chamber. A class 8 clean room is provided, including a dry heat steriliser. It is planned to upgrade parts of this facility to a high clean room class (3 or better) in order to develop ultra cleaning processes space and terrestrial applications (e.g. for support of life detection missions to Mars).

This unique facility combination does offer academia and industry a modern laboratory and the possibility to perform experiments or verification activities which are beyond standard test capabilities. The LPS further offers the expertise and the facilities to perform assessments and experimental verifications of instrument design concepts. The LPS and its team can support the science community and industry e.g. in 'Rapid Bread Boarding', experiment design as well as in experiment preparation and execution. Furthermore, specific analysis in the fields of the team's expertise can be performed on demand. This facility is also equipped to host science verification and flight sequence tests (http://www.esa.int/esaHS/SEM3E813J6F_research_0.html) and biological, physical or long term functional tests of flight-instruments and ground reference-models.

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Symposium III

The ISS as a National Lab

Chair: Ken Souza

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[55]

USE OF THE INTERNATIONAL SPACE STATION AS A US NATIONAL LABORATORY. M.L. Uhran, NASA Office of Space Operations, Washington, DC.

The International Space Station (ISS) is scheduled to complete assembly by the end of 2010. At that stage, it will begin operating as a US national laboratory, and become available for use by US government agencies, private firms and non-profit institutions. As ISS assembly approaches completion and next-generation space transportation systems begin to emerge, the perceived cost and schedule risks associated with research in space are diminishing. NASA has already entered into several memoranda of understanding and Space Act agreements for this purpose, and further research partnerships are anticipated in the future. A review of progress to date on the ISS National Laboratory initiative reveals principle areas of scientific, technological and industrial interest that are being driven by contemporary research themes. Since the ISS was initially designed to be a flexible laboratory environment, it is well positioned to host these emerging areas of investigation. The experiences in space-based microgravity laboratory research over the last few decades also contribute strongly to understanding the ingredients necessary to successful research partnerships and proposals for the ISS era.

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DISCOVERY OF SPACEFLIGHT-REGULATED VIRULENCE MECHANISMS IN *SALMONELLA* AND OTHER MICROBIAL PATHOGENS: NOVEL APPROACHES TO COMMERCIAL VACCINE DEVELOPMENT. C.A. Nickerson¹, J.W. Wilson², C.M. Ott³

¹The Biodesign Institute, Center for Infectious Diseases and Vaccinology, Arizona State University, ²Department of Biology, Villanova University, and ³Habitability and Environmental Factors Division, NASA-Johnson Space Center.

Understanding infectious disease risks during spaceflight is critical to provide safe passage for human exploration to the moon and Mars and holds exciting potential for innovations in infectious disease control for the general public on Earth. The key to this research is the novel way that cells adapt and respond to spaceflight, as they exhibit important biological characteristics that are directly relevant to human health and disease including changes in immune function, cellular stress responses, virulence and infectious disease potential that are not observed using traditional experimental approaches. We previously discovered that spaceflight uniquely alters the virulence and gene expression of the bacterial pathogen *Salmonella typhimurium*, and that the conserved, small regulatory RNA-binding protein Hfq plays a central role in regulating the *Salmonella* spaceflight microgravity response. Hfq is a highly conserved bacterial RNA chaperone protein that plays a diverse role in prokaryotic gene expression, virulence, and physiology in response to stress. We have subsequently shown that spaceflight culture of *Pseudomonas aeruginosa* also alters the *hfq* regulon in this bacterial pathogen. As Hfq regulation is often associated with ionic salt concentrations, we investigated and discovered that altering the concentration of certain ionic salts, like phosphates, in the growth media can prevent the increased disease causing potential observed for *Salmonella* during spaceflight. Collectively, our results suggest that RNA binding regulatory proteins and their small RNA binding counterparts may be key to a conserved, common cellular spaceflight response mechanism in bacterial cells, and that this response can be manipulated by environmental salt/ion levels. The implications of an evolutionarily conserved molecular response to spaceflight would affect NASA's approach to infectious disease risk assessment, development of biological processing systems for exploration, and other mission-related functions. Importantly, the knowledge gained from this work will broaden our knowledge of microbial cells for both spaceflight and Earth based applications. (Supported by NASA NCC2-1362).

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INTERNATIONAL SPACE STATION RESEARCH AND FACILITIES FOR LIFE SCIENCES. J.A. Robinson, T.M. Ruttley and C.A. Evans

Office of the ISS Program Scientist, International Space Station Program, NASA Johnson Space Center, Houston, TX

Assembly of the International Space Station is nearing completion in fall of 2010. Although assembly has been the primary objective of its first 11 years of operation, early science returns from the ISS have been growing at a steady pace. Laboratory facilities outfitting has increased dramatically 2008-2009 with the European Space Agency's *Columbus* and Japanese Aerospace Exploration Agency's *Kibo* scientific laboratories joining NASA's *Destiny* laboratory in orbit. In May 2009, the ISS Program met a major milestone with an increase in crew size from 3 to 6 crewmembers, thus greatly increasing the time available to perform on-orbit research. NASA will launch its remaining research facilities to occupy all 3 laboratories in fall 2009 and winter 2010. To date, early utilization of the US Operating Segment of the ISS has fielded nearly 200 experiments for hundreds of ground-based investigators supporting international and US partner research. With a specific focus on life sciences research, this paper will summarize the science accomplishments from early research aboard the ISS- both applied human research for exploration, and research on the effects of microgravity on life. We will also look ahead to the full capabilities for life sciences research when assembly of ISS is complete in 2010.

[58]

GENETIC MEDIATORS OF *SALMONELLA* VIRULENCE DURING SPACEFLIGHT IN A NEMATODE MODEL.

T.G. Hammond¹, J. L. Becker², A.L. Johnson³, J.S. Hammond³, M.A. Gunter⁴, L.S. Stodieck⁵, and P.L. Allen¹. ¹Durham VA Medical Center and Duke University, Durham, NC, ²National Space Biomedical Research Institute, & Baylor College of Medicine, Houston TX, ³Institute for Medical Research, Durham NC, ⁴Department of Health, Macon GA, and ⁵Bioserve Space Technologies, University of Colorado, Boulder, CO

Salmonella infects the nematode *Caenorhabditis elegans*, yielding a model system to test the molecules which determine virulence of *Salmonella* strains in both the infecting bacteria and the nematode host. Wild type and Tol1 deletion nematode eggs and adult worms were exposed during spaceflight, or in ground clinostat or static cultures to *Salmonella*, with and without specific gene deletions. Use of multiple chamber glass tubes with pass through valves (Bioserve Fluid Processing Apparatus) allowed preparation of bacteria, nematodes and fixative in separate chambers prior to launch. On-orbit mixing of nematodes and bacteria was performed 20 hours following launch; 48 hours later experiments were terminated by the addition of fixative. For the first time ever, direct study of an inflight infection model using nematodes and *Salmonella* demonstrates: (1) under the conditions we utilized, *Salmonella* grows at the same rate in static or clinostat ground cultures as in spaceflight; (2) *Salmonella* increases in virulence during both spaceflight and clinostat culture; (3) deletion of pathogenicity island 3- or 5-, but not pathogenicity island-1, genes reduces *Salmonella* virulence during spaceflight; (4) changes in *Salmonella* virulence during space flight are dependent on the Tol1 gene in the nematode host; and (5) the increased virulence of *Salmonella* during clinostat culture is mediated by different gene pathways than the virulence effects induced in spaceflight.

(Supported by SpaceHab/Astrotech Inc. & Durham VAMed. Ctr.)

Oral Session III

Space Biology and Physiology: Animals, Cells and Microbes

Chair, David Tomko

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THE PHARMASAT NANOSATELLITE PLATFORM FOR LIFE SCIENCE EXPERIMENTATION: EFFECTS OF SPACE FLIGHT ON ANTIFUNGAL ACTIVITY AGAINST *SACCHAROMYCES CEREVISIAE*. M. Parra¹, D. Ly¹, A.J. Ricco², M.R. McGinnis^{3,4} and D. Niesel³. ¹Lockheed Martin Mission Services and ²NASA Ames Research Center, Moffett Field, CA, ³Dept. of Microbiology and Immunology and ⁴Pathology UTMB, Galveston, TX.

Small satellites offer enormous potential for future space life sciences research in terms of lower costs and greater access to space. These fully autonomous “free-flyers” are equipped with multiple sensors to report the status of the satellite and its biological payload. As secondary payloads, small satellites can be integrated onto a variety of launch vehicles having minor extra mass margins. With this greater access to space comes the need for early payload integration. Therefore, organisms and reagents must be rigorously tested to ensure survival in a state of stasis at ambient temperatures for long time periods—several to many weeks—before experiment initiation. PharmaSat, the second successful biological nanosatellite and the first with a PI-led science experiment, was designed to test the effect of microgravity on yeast susceptibility to antifungal drugs. To accomplish this, the forty-eight 100- μ L wells of a microfluidic card were loaded with different population densities of the yeast *Saccharomyces cerevisiae*. The yeast cells were activated with growth medium when the satellite attained stable orbit. After recovery from prolonged stasis, cells were exposed to three concentrations of the antifungal Voriconazole; a zero-concentration control was also included. PharmaSat was launched on May 19, 2009, and the experiment activated ~48 hours post-launch. All systems on the satellite performed nominally; temperature, pressure, humidity, and power stayed within expected ranges. Furthermore, communication was established with the satellite on the first attempt and payload health information as well as science data was downloaded successfully throughout the operational mission phase. Science results show dependence of growth parameters on antifungal concentration in both ground and space-flight experiments. (Supported by: Grant/Coop. Agreement #: NNX07AD28A S05, and NASA Exploration Systems Missions Directorate (ESMD) ISS Non-Exploration Research Project)

[61]

SPACEFLIGHT EFFECTS ON GENE EXPRESSION AND EXOTOXIN A PRODUCTION IN *Pseudomonas aeruginosa*. B.H. Pyle, S.C. Broadway, K. McInerney, K. Williamson. Dept. of Microbiology, Montana State Univ., Bozeman, MT.

Two strains of *Pseudomonas aeruginosa* were flown in the Microbial Drug Resistance and Virulence (MDRV) experiment on Shuttle Endeavour STS-123 in March, 2008. The objectives were to assess spaceflight effects on virulence, physiology and RNA gene expression of this opportunistic pathogen. Cultures were loaded in Fluid Processing Apparatus containers housed in Group Activation Packs. PA01, ATCC BAA-47, and PA103 ATCC 29260, a high Exotoxin A (ETA) producer, were grown in two broth media: PA01 in Lennox broth (LB) and both PA01 and PA103 in MSDM2 broth for ETA production. On-orbit cultures and ground controls were incubated at ambient temperatures for 25h (PA01 in LB) or 51.5h (MSDM2 cultures). Growth in cell numbers was similar for all cultures, and those in LB were probably in late log phase and MSDM2 in stationary phase at termination. No differences were detected in Exotoxin A (ETA) production for either strain from flight vs. ground incubated cultures. RNA gene microarray analyses showed that, overall, 348 genes were either up- or down-regulated. Two- or three-fold more genes were down-regulated than up-regulated in flight vs. ground cultures. Genes that were down-regulated in both strains in MSDM2 included mainly unknown genes or those coding for hypothetical proteins. Other genes up-regulated in strains PA01 and PA103 grown in MSDM2 included those related to iron metabolism, RNA synthesis and energy metabolism. Since this medium is limited in iron, it might be expected that iron metabolism and scavenging genes might be up-regulated in the microgravity environment that limits convection and diffusion. Of the three variables (strain, medium, and spaceflight), the medium had the most effect followed by the strain and spaceflight. This suggests that the selection of microbial strains and media is critical to obtaining comparable results for spaceflight experiments. (Supported by NASA: NAS2-1143, Ames Research Center.)

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SPACE FLIGHT ALTERS *STREPTOCOCCUS PNEUMONIAE* GENE EXPRESSION AND VIRULENCE ACTIVITY. U. Pandya^a, R. Carmichael^b, D.A. Watson^{a,c,d,e}, N. Williams^a, K. Sato^f, H.E. Ray^f, D. W. Niesel^g ^aDepartment of Microbiology and Immunology^a, Biochemistry and Molecular Biology^b, UTMB, Galveston, TX; ^cNSBRI^c, Ctr for Space Med^d, BCOM^e, Houston, TX; ^fLMMMS, ARC^f, Moffett Field, CA

Streptococcus pneumoniae is an opportunistic bacterial pathogen that can mediate significant disease and is a commensal inhabitant of the human nasopharynx. *S. pneumoniae* cultures were initially flown on STS-118 using the SPEGIS Canister Assembly, grown on the Orbital Middeck using the Microgravity Environment Research Locker/Incubator (MERLIN), stowed frozen on the ISS using the -80 laboratory freezer (MELFI) and compared to synchronous ground cultures upon return. Comparison between flight and ground control cultures revealed differences in growth and in the activity of the virulence factors - neuraminidase and β -galactosidase. Examination of the total proteome by 2-D PAGE revealed 17 protein spot intensity differences with 13 proteins showing enhanced expression (range 1.9 – 2.7 fold; 27.8kDa to 112kDa) and 4 showing reduced expression (range 1.7 – 5.6 fold; 14kDa to 121kDa). Microanalysis showed 104 differentially-expressed genes representing most functional groups and genome locations. *S. pneumoniae* cultures were later flown on STS-123 as part of the MDRV using the Bioserve Group Activation Pack (GAP) and fluid processing apparatus (FPA). The cultures were maintained on the Orbital Middeck at ambient temperature. Flight and synchronous ground control cultures were used for mouse challenge. A clear difference was observed in mouse virulence between the flight and ground control cultures in the LD₅₀ (>10-fold decrease). This adds to accumulating information suggesting that pathogenic bacteria alter gene and protein expression as they adapt to the space environment and that these changes alter virulence potential. NASA– 1D1 USRA 98-HEDS 02-294

[62]

SOYUZ/ISS KUBIK-EXPERIMENT XENOPUS: EVIDENCE FOR G-RELATED CRITICAL PERIODS IN AMPHIBIAN DEVELOPMENT. E.R. Horn and M. Gabriel. Gravitational Physiology, Ulm University, Ulm, Germany

During the Soyuz flight TMA13 (upload) and TMA12 (download) in October 2008, tadpoles were exposed to microgravity for about 10 days. The experiment XENOPUS was the 4th in a row of space flight experiments performed in 1993, 1997 and 2001 dedicated to the analysis of microgravity effects on the development of *Xenopus laevis*, in particular of its roll-induced vestibuloocular reflex (rVOR). For the recent flight, 2 tadpole stages were selected according to the development of their legs: stage 47 (flight: n=20; ground n=40) had just formed their hind limb buds, and stage 50 (flight n=16; ground n=32) had just formed the forelimb buds. Parent animals for each stage differed, but flight and corresponding ground tadpoles had the same parents. From the 36 Soyuz/ISS tadpoles, 35 survived, from the 72 ground controls 64. Spontaneous swimming behaviour was recorded 1 hour after touch-down of Soyuz at the landing site. Recordings of the rVOR started 19 hours later. In June 2009, observations about general development (stage, body size, and distance between the eyes) are still running. - Main observations: (1) One hour after landing, stage 47 tadpoles revealed looping swimming, while stage 50 tadpoles swam normally. All ground controls swam normally. (2) Microgravity induced tail lordosis dominated in stage 47 tadpoles (14 out of 19) compared to stage 50 tadpoles (1 out of 16). All ground controls developed straight tails. (3) The rVOR was not affected by microgravity exposure in both tadpole groups compared to their ground controls. (4) Post-flight growth was significantly affected by the space flight; in the younger tadpole group, flight animals grew faster compared to their ground controls. - Conclusions: These observations and results obtained from former experiments on STS-55, STS-84 and Soyuz TM33/TM32 (Horn and co-workers) and on STS-47 (Souza and co-workers) with *Xenopus* allow the definition of critical periods for the development of the rVOR and the induction of tail lordosis. - Financial support by DLR, grant 50WB0630.

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EARLY RESULTS FROM BIOLOGICAL STUDIES ABOARD THE JAPANESE EXPERIMENT MODULE “KIBO” OF THE ISS. M.

Takaoki, N. Fujimoto, S. Ogawa, and T. Nakamura. Space Environment Utilization Center, Japan Aerospace Exploration Agency, Tsukuba, Japan. The Pressurized Section of Japanese Experiment Module “Kibo” became operational in August 2008, and biological studies started early 2009. The onboard operations for three themes, Rad Gene, LOH and Dome Gene, have been successfully completed. Rad Gene is a study into the functions of the tumor suppressor *p53* gene in space. Normal and abnormal *p53* mutant cells with human lymphoid origins were launched frozen and cultured aboard “Kibo” certain duration, then retrieved frozen. The expressions of genes under the influence of *p53* are analyzed referring to the exposure to the space radiation. LOH is detect chromosomal damage by space radiations efficiently utilizing the loss of heterozygosity. Human lymphoid cells heterozygous for thymidine kinase, launched frozen, were cultured aboard “Kibo”. The frozen and retrieved cells were cultured on the ground with a selection medium to detect thymidine kinase negative, i.e. damaged and lost the heterozygosity, cells in high sensitivity. Dome Gene is to study the morphogenetic mechanism of the tissue stereoisomeric structure. A6 cells derived from *Xenopus* renal epithelial tissue, which form dome structure under normal gravity, were cultured aboard “Kibo”. The formation of dome structure under different gravitational conditions were analyzed in relation to the gene expression profiles. Studies with *Arabidopsis*, *C. elegans*, Silkworm as well as mammalian cells are scheduled to follow.

[65]

EFFECTS OF PARABOLIC FLIGHT ON SEROTONIN-RELATED GENE EXPRESSION IN THE MOUSE BRAIN

M. Yoshioka¹, T. Yamaguchi¹, H. Ohta², J. Gyotoku³, and T. Ochiai³
¹Department of Neuropharmacology, Hokkaido University Graduate School of Medicine, Sapporo, Japan, ²Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., Japan, ³Mitsubishi Heavy Industry, Kobe, Japan

A number of parabolic flight experiments have shown that processes within the central nervous system (CNS) are affected by weightlessness in human. It is, however, likely that changes in the CNS observed during parabolic flights are not solely due to the repeated changes in gravity experienced during the flight. Instead, these changes may be related to secondary psycho-physiological reactions to the emotional and physical stress during these flights. The purpose of the present experiment is to elucidate whether gene expression levels, especially serotonin-related genes, are altered in the mouse brain exposed to gravity-changing stress using a RT-PCR method. Mice were exposed to gravity-changing stress during 8 times repeated by parabolic flights performed by an airplane. Serotonin transporter, tryptophan transporter and tryptophan hydroxylase-2 mRNA levels in the midbrain 6 h after the flight were significantly increased compared with pre-parabolic flight control. In contrast, those of monoamine oxidase-A, 5-HT_{1A} receptor and GAD65/67, synthases for GABA, were not altered by the flight. The results of the present study suggest that the serotonergic system, particularly synthetic pathway, might be activated in the CNS by gravity-changing stress.

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EFFECTS OF SPACE FLIGHT ON MOUSE GRANULOCYTIC BONE MARROW CELL POPULATIONS. S.K. Chapes¹, M.T.

Ortega¹, M.J. Pecaut², D.S. Gridley², V. Ferguson³ and L.S. Stodieck³,
¹Div. of Biology, Kansas State Univ., Manhattan, KS, ²Dept. of Radiation Medicine, Loma Linda Univ., Loma Linda, CA, ³Dept of Aerospace Engineering, BioServe Space Technologies, Univ. of Colorado, Boulder, CO.

We isolated bone marrow cells from C57BL/6J mice following a 13-day flight on STS-118. We used flow cytometry to assess the expression of molecules that define the maturation/activation state of cells in the granulocytic lineage on three bone marrow cell subpopulations. These molecules included Ly6C, CD11b, CD31 (PECAM-1), Ly6G (Gr-1), F4/80, CD44 and c-Fos. We focused on three subpopulations of bone marrow cells including small agranular cells (R1), larger granular cells (R2) which were mostly neutrophils and very large, very granular cells (R3) which had properties of macrophages. We found that there were subpopulation differences in Ly6C (R1 and R3), CD11b (R2), CD31 (R1, R2 and R3), Ly6G (R3), F4/80 (R3), CD44 high (R3), and c-Fos (R1, R2 and R3). CD11b was elevated in the R2 subpopulation suggesting a neutrophil response to landing. We also detected decreases in Ly6C, c-Fos, CD44^{high} and Ly6G and an increase in F4/80 in the R3 subpopulation. These data suggest that the bone marrow cells in the R3 subpopulation of space flight mice were more differentiated compared to the ground controls. Over all, these data suggest that there are significant changes in bone marrow cell phenotype in response to the stress of the space flight experience.

This project was supported in part by NASA grant NAG2-1274, by the NASA space grant consortium, NIH grants AI55052, AI052206, RR16475, RR17686, the Kansas Agriculture Experiment Station, the Terry C. Johnson Center for Basic Cancer Research and by the Department of Radiation Medicine LLURM Molecular Radiation Biology Laboratories.

[66]

DEFINITION OF MULTI-INVESTIGATOR COMPATIBLE TISSUE RETRIEVAL OF MALE AND FEMALE REPRODUCTIVE ORGANS TO AID IN BIOSPECIMEN SHARING PROGRAM PROCEDURES FOR ACUTE POST-FLIGHT TISSUE COLLECTION.

V. Gupta^{1,2}, L. Holets^{1,2}, K.F. Roby, J.S. Tash^{1,2}, U54 Interdisciplinary Center for Male Contraceptive Research & Drug Development, ²Dept. Mol. & Integr. Physiology, Univ. Kansas Medical Center, Kansas City, KS,

With the paucity of flight opportunities for mammalian physiology in space flight, the Biospecimen Sharing Program (BSP) for acute post-flight tissue collection offers the opportunity to maximize the number of PI's and the data generated from a flight. However, the logistics of multiple tissue collection teams during post-flight processing necessitates determination of the limits of time and temperature between euthanasia and fixation or RNA extraction to ensure stable histology and RNA derived data. STS-131 and BION M1 will be flying combinations of male and/or female mice and gerbils for 12d up to 35 d in orbit. The aim of our study was to determine the windows of time between euthanasia and tissue harvest that will yield no significant impact on resulting histology, and subsequent sperm morphology and motility analysis of live sperm retrieved from the epididymis. We euthanized 6 mice each and retrieved testis and epididymis (at 0, 0.5, 1, 1.5, 2 and 2.5hr post-euthanasia) or ovaries and uteri (at 0, 0.5, 1, 2 and 3hr post-euthanasia). Our results indicated that in male C57Bl/6J mice, testicular histology and epididymal sperm motility remain largely unchanged when tissue harvest was delayed up to 3 hr after euthanasia. There was no change in the sperm motility or testicular histology when tissues from all mice were retrieved but stored at room temp. or on ice before analysis at the above time intervals. In female mice, histology of ovaries and uteri retrieved up to 3 hrs post-euthanasia remained unchanged. As we prepare for BSP functions on STS-131 and the BION M1 mission, we have determined the windows of time, and temperature for optimal processing of both male and female tissue preservation (testis, sperm, ovary, and uterine horns) for morphology. This study will aid in designing the reproductive tissue collection regimen from live animal models for any project especially those involving multiple investigators. Finally, our protocols for reproductive tissue harvest are compatible with investigators who require more rapid tissue retrieval.

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METHODS FOR SAFE POST-FIXATION PROCESSING AND SHIPMENT OF MICE TESTICULAR SAMPLES. ^{1,2}LM Holets, ^{1,2}V Gupta, ^{1,2}JS Tash, ¹Interdisciplinary Center for Male Contraceptive Research & Drug development, ²Dept. Mol. & Integr. Physiology, Univ. Kansas Med. Center, Kansas City KS.

Our participation in the STS-131 and BION M1 Biospecimen Sharing Programs requires tissue harvest post flight at KSC and the IMBP labs in Moscow, then shipping the samples back to our lab in the US for final processing/data collection. Testes are particularly complex tissues that require a strong fixative such as Bouin's solution to retain histology. However, safety requirements prevent tissue samples from being shipped back to the PI's labs in fixative or alcohol solutions. Thus, we report here new methods for 1) fixative removal into aqueous media for safe shipping, as well as 2) reconstitution protocols into 70% that retain excellent histology. The histological modifications that occurred in testis and epididymis after replacement of flammable 70% ethanol (ETOH) to safe PBS have been compared. Testis and epididymis from 12 mature mice (C57Bl/6) were harvested. Tissue were fixed in Bouin's solution for 48 hrs, washed in 70% ETOH, and divided into 4 groups. In group 1 (control), the tissue was store in 70% ETOH until paraffin embedding. In groups 2-4, 70% ETOH was substituted with PBS (pH=7.4) for one wk, and then rapidly or subsequently replaced with 10%, 30%, 50%, 70% ETOH. Tissue was paraffin-embedded and processed for histology. Testicular morphology was evaluated for histological changes. The step-wise replacement of ETOH-PBS-ETOH caused less destruction of tissue then a single-step change of solution. Testis was more sensitive to dehydration/rehydration shock than epididymis. Total RNA was isolated from testis with safe RNAlater solution (Ambion) vs TRIzol reagent. Our data demonstrate high RNA quality and stability of spermatogenesis important genes expression in tissue stored in RNAlater solution. We have developed a method for post-fixation processing of tissues at KSC or Moscow IMBP labs that removes fixative and alcohol for safe shipment back to our laboratory and subsequent re-dehydration and processing for retention of excellent histology. This method should be evaluated for use on other tissues to maximize optimal histology and gene transcription data collection in the primary flight experiments as well as the BSP investigators.

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ALTERED GRAVITY EXACERBATES CHROMATE-INDUCED GENOTOXICITY. John Pierce Wise Jr.^{1,2}, Sandra S. Wise^{1,2,3}, James Wise^{1,2}, Jane McKay^{1,2}, Michael Browne⁴, Kellie Joyce^{1,2,3}, Matthew Braun^{1,2}, Catherine Wise^{1,2}, Ryan Duffy^{1,2}, Eben Estell⁴, Jennifer Brown⁴, Christy Gianios Jr.^{1,2}, Michael Mason⁴, Terry Shehata⁵, Dianne Hammond⁶ and John Pierce Wise Sr.^{1,2,3}

¹Wise Laboratory of Environmental and Genetic Toxicology, ²Maine Center for Toxicology and Environmental Health, ³Department of Applied Medical Sciences, University of Southern Maine; ⁴Department of Chemical and Biological Engineering, University of Maine; ⁵Maine Space Grant Consortium; ⁶NASA Johnson Space Center, Houston, TX

CONSTELLATION is NASA's next mission to explore the surface of the moon. Under this program, NASA plans to send manned missions back to the Moon by the year 2020. Thus it is essential to determine the effects of altered gravity on cellular morphology and metabolism to make long-term space travel safer for the astronauts involved. We hypothesized that altered gravity changes normal cell function resulting in an increase in chemical-induced genotoxicity. We conducted our experiments aboard NASA's Weightless Wonder, a C9-B plane that simulates environments of microgravity (0 g) and hypergravity (2 g). We exposed human lung fibroblast cells to sodium chromate during flight along with a parallel experiment conducted simultaneously on the ground. We found that, after a 4 h exposure, altered gravity increased the amount of chromosomal damage. We further found that altered gravity decreased the amount of chromium ion uptake indicating that differential uptake was not the underlying mechanism. These data indicate that altered gravity can significantly increase the potency of genotoxic agents, which suggests that risks of exposure to astronauts in space are greater than on earth. Future research is aimed at understanding the relative contributions of hyper- and microgravity to these effects and investigating the underlying mechanisms. This project was primarily supported by the Reduced Gravity Flight Opportunities Program at the Johnson Space Center and the Maine Space Grant Consortium.

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