Future Opportunities for Life Science Programs in Space

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Key Words:
Space biology
Microgravity
Amphibian embryogenesis
Research opportunities
NASA
NIH
NSF

Most space-related life science programs are expensive and time-consuming, requiring international cooperation and resources with trans-disciplinary expertise. A comprehensive future program in "life sciences in space" needs, therefore, well-defined research goals and strategies as well as a sound ground-based program. The first half of this review will describe four key aspects such as the environment in space, previous accomplishments in space (primarily focusing on amphibian embryogenesis), available resources, and recent advances in bioinformatics and biotechnology, whose clear understanding is imperative for defining future directions. The second half of this review will focus on a broad range of interdisciplinary research opportunities currently supported by the National Aeronautics and Space Administration (NASA), National Institute of Health (NIH), and National Science Foundation (NSF). By listing numerous research topics such as *alterations in a diffusion-limited metabolic process, bone loss and skeletal muscle weakness of astronauts, behavioral and cognitive ability in space, life in extreme environment*, etc., we will attempt to suggest future opportunities.

Designing appropriate, cost-effective experiments will be conceptually and technically difficult, since microgravity effects can be subtle and therefore difficult to recognize (Schmitt, 1999). In order to help identify future research opportunities in space, we will first describe the interactive aspects of life sciences in space by addressing the following four questions:

- What are true microgravity effects?
- Which basic biological phenomena can be better understood using a microgravity environment?
- What technical advantages does a space station offer?
- Which commercially viable products can be produced in a microgravity factory?

The space environment

Understanding the environment in space is a first step for predicting the future directions of microgravity life sciences. It is important to note that a microgravity effect, observed in space (e.g., random orientation of axis of growth of a higher plant during spaceflight), is not necessarily identical to unloading effects observed on ground (e.g., muscle atrophy in bed ridden patients). Furthermore, micro-gravity effects need to be distinguished from other physical effects in space. Spacecraft liftoff forces, radiation levels (Suzuki, 1998, Kiefer and Pross, 1999), ambient atmospheric conditions (Musgrave et al., 1997), and temperature extremes come into play during the typical spaceflight experiment and need to be dealt with, but not as microgravity effects. Some research projects are specifically designed to elucidate the effects of microgravity on living systems. Such research projects can be viewed either from the basic science viewpoint of gaining a better understanding of life's origins, mechanisms, and strategies (Boonstra 1999), or from the practical viewpoint of improving astronaut health and/or colonizing outer space (Helick 1998). Recent studies suggest that cells may sense gravity through changes in the balance of forces that are transmitted across transmembrane adhesion receptors (Ingber, 1999). Other research projects are designed to exploit microgravity as a tool to answer fundamental questions about cells, tissues, and organs. The unique environment of microgravity will eventually be available as yet another "laboratory condition" for studying basic aspects of the structure and function of biological macromolecules (Yokota et al., 1998).

Previous accomplishments in space

Focusing mainly on amphibian embryogenesis in space, we will describe previous accomplishments in space. To broaden the search for *true* microgravity effects, substantial attention has been given to a variety of

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embryos. The concept being that the *inherent fragility of the embryo would provide an excellent test system for recognizing even subtle microgravity effects.* Even small microgravity effects might cascade into major, catastrophic effects as growth of the embryo magnified small defects into easily recognized monstrosities. This idea is especially appealing because it would likely offer an opportunity to recognize individual cell types as microgravity targets (Asashima et al., 1999).

Amphibian eggs have provided the best test system, for not only are they vertebrates, but they have been fertilized during spaceflight, permitted to develop through the early stages of embryogenesis, and then examined upon return to Earth. Two completely independent experiments, employing different species (frog/newt) have generated essentially similar data (Sousa et al., 1995; Mogami et al., 1996). Amphibian eggs were fertilized and the embryos developed normally through the organ and tissue development stages under microgravity. A number of subtle effects were observed: (i) the location of the first horizontal cleavage furrow was shifted toward the vegetal pole at the eight-cell stage, (ii) the position of the blastocoel was more centered, (iii) the dorsal lip appeared closer to the vegetal pole. Similar effects were observed under the simulated unloading on ground by clinostation (Neff et al., 1993). It would be interesting to investigate whether self-correcting mechanisms such as re-targeting of neurons or compensatory growth of an affected tissue takes place in amphibian embryogenesis under microgravity. It is possible that future space trials will reveal more subtle effects at one or another level of biological organization.

For a vertebrate, the "dream" experiment will be testing egg-to-egg development. In the case of amphibians, the difficulty in providing long-term (6 months to one year for a "generation time") husbandry for eggs, embryos, larvae, metamorphosing animals, and adults preclude such experimentation in the near future. Jellyfish embryos have also been analyzed for spaceflight effects. Although many embryos appeared to be normal, some embryos showed various presumed microgravity effects on limb development and swimming behavior (Spangenberg et al., 1997).

Available resources

Besides Earth-based support facilities, a space station will be available in the 21st century. Although many life scientists are skeptical about the usefulness of the space station (Reichhardt et al., 1998, Lawler, 2000), several advantages are easily recognized: experiments for long duration (which might permit for some animal species egg-to-egg embryogenesis), relatively large onboard centrifuges for performing appropriate control experiments, more sophisticated (and larger) equipment for specimen manipulation, and more appropriate measuring devices for data collection. The use of a centrifuge control will be included in the experiments on the space station. Since any *true* microgravity effects will likely be subtle, and therefore difficult to quantify or otherwise document, data from onboard, centrifuged specimens will be a prerequisite for drawing conclusions about putative microgravity effects. To date, due to technical constraints of spacecraft design (limited space) and flight duration (typically only several days) most space biology experimentation has employed ground-based specimens as controls.

Several projects have been developed with commercialization as a driving force. For example, the production of large protein crystals, which would facilitate elucidating the molecular structure of potential intracellular drug targets, has been one important goal. Improved quality of protein crystals has been reported in microgravity (Littke and John, 1986, Berisio et al., 2000). However, high costs challenge the commercial potential of this space research platform. The prospects for direct commercial-scale production in a microgravity environment and a possible bio-technology factory inside a space station wait for creative cutting-edge bio-technology research (Canizares, 1998, Klaus, 1998).

Advances in bioinformatics and biotechnology

Recent rapid advances in bioinformatics and biotechnology have the potential to reshape future life sciences in space. The genome of many organisms including *C. elegans* (Bargmann, 1998), *Drosophila* (Sanchez et al., 1999), mouse (Blake et al., 1999), and human (Collins, 1998) will soon be fully sequenced. It is probable that *virtual gene searches* using computational tools will provide insights into potential micro-

Table 1. NASA-sponsored research opportunities in space life sciences

High priority area	Research subject
Molecular structures and physical interactions	Static boundary layer effects on gas exchange Changes in heat transfer Lack of convective fluid movements Alterations in diffusion-limited metabolic processes
Developmental biology	Gametogenesis and fertilization Pattern formation Organogenesis Neural and vestibular development Development of gravisensing systems
Cellular and molecular biology	Cell cycle Apoptosis Cell senescence Cell growth Nerve cell formation Response to injury in nerve cells
Organismal and comparative biology	Response to gravitational force Effects of hypergravity and hypogravity Regulation of physiological systems Interaction of gravity and other environmental factors
Gravitational ecology	Structure and function of ecosystem Evolution or stability of ecosystem

Table 2. NIH program titled "Earth-Based Research Relevant to the Space Environment"

Research area	Research subject
Spatial orientation and sensory-motor processes	Role of binaural auditory cues Development of the vestibular system Central adaptation of vestibular functions Vestibular autonomic regulation and dysregulation Spatial human cognition Sensory structures and neural pathways Adaptation of sensory-motor systems Eye movement Postural control Spatial navigation Sensory-motor integration
Nervous system	Autonomic control of blood pressure Development of the central nervous system Cerebral homeostasis Neuronal plasticity Gene transfer Neuroendocrine function Chemosensory changes
Behavioral and psychological processes	Technique to evaluate behavior Neurobiological and psychosocial mechanisms Effects of the space fight environments Space flight associated stresses Affective and cognitive responses Strategies during long-duration missions Influence of crew psychosocial heterogeneity Effects of differences in the cultures
Cardiovascular function	Biophysics of cell signaling Arterial, systolic, and diastolic blood pressure Vascularity of muscle fibers Vascular tone and responsiveness Cerebral cortical blood flow Time-dependent development of high blood pressure Role of salt sensitivity or sympathetic nerve activity Cardiovascular adaptations Autonomic function Peripheral vascular changes Vestibular/autonomic nervous system interaction Interaction with circadian rhythms
Musculoskeletal system	Microgravity-induced osteopenia and muscle atrophy Mechanisms to external loading and unloading Turnover of matrix and contractile proteins Bone loading Bone loss and skeletal muscle weakness Effects of hormonal and growth factor Cellular response to altered stress and gravity Evaluation of 3D structure and integrity Gene expression of bone and muscle cells Alterations in blood flow Development of therapeutic agents Motor unit loss Role of exercise and overload Trophic factors Role of nutrition on muscle mass Effect of microgravity, bed rest, and aging Effects of changes in connective tissue Role of neural vs. muscular changes
Musculoskeletal system	Comparison of the effects of aging Effects on older vs. younger men and women Interactions with other systems Changes in calciotropic hormones Uncoupling of bone remodeling Enhanced propensity for falls and fractures Lighting and Vitamin D synthesis Bone density Bone cell function and activity Nutritional and environmental factors to bone Countermeasures to bone loss Chemical signals on bone growth Vascularity of muscle fibers Physiologic changes of bone and skeletal muscle
Pulmonary function	Progression of pneumonia and other infections Cellular processes across lung alveolar cells Defense mechanisms Oxygen radical formation and antioxidant activities Expression of differentiation-specific genes Effects on various lung cells Pulmonary microvascular permeability changes Pulmonary vascular tone and responsiveness Neurobiological transduction proerties Mechanisms of force reception in lung cells
Sleep and biological rhythms	Long and short-term adaptations Periodic breathing, dyspnea, and sleep apnea Age effect Changes in circadian/sleep cycles Neurobehavioral deficits Behavioral and pharmacological interventions
Immunology	Immunological change Initiation of immune dysregulation and reversibility Responses to opportunistic infection Immune responses and neuroendocrine parameters Cellular and animal models of disease
Pharmacodynamics and pharmacokinetics	Physiological sequelae of medication Behavioral and cognitive ability Pharmacokinetics of pharmacological compounds Drug delivery
Hemodynamics	Innovative mechanisms affecting gene expression Regulation of hemoglobin gene expression Regulation of globin gene expression Formation of platelet micro-aggregates and activation Storage of platelets
Injury	Fluid and cardiovascular resuscitation Physiology of shock and compensatory mechanisms Routine clinical assessments Bioengineering principles Acute to long-term responses to injury Initiation of and response to infection Common complications of patients Wound healing and tissue repair Tissue engineering Psychological, emotional, and cognitive aspects

gravity effects. Large numbers of gene sequences, or batteries of known genes will be assayed in tissues and organs for alterations in expression patterns under microgravity.

One example is a gene involved in circadian rhythms. Natural selection has favored in nearly all living organisms circadian rhythmicity that persists with an intrinsic period close to that of Earth's rotation (Czeisler et al., 1999). Clock genes, responsible for circadian oscillations, have been identified in plants, insects, and mammals (Tei et al., 1997). Circadian clocks are altered during spaceflight, and it will be straightforward to test the function of a particular gene in regulating circadian rhythms by determining the mRNA or protein products produced under microgravity conditions. A start in this direction has been achieved, and the interaction of transcription factors with promoter sequences appears to be a major regulatory step in response to microgravity (Moore, 1999, Ikenaga et al., 1998). We expect that it will soon be possible to determine gravity effects on the expression of many other specific genes.

Table 3. NSF-sponsored program titled "Life in Extreme Environments"

Theme	Research subjects
Microbial systems	Microorganisms in extreme environments Possible utility in biotechnology Identification of unusual or unique chemical compounds Paleobiological studies
Extreme environments on Earth	Significant examples of extreme environments Physical, chemical, biological, and geological processes
Planetary environments	Formation of planets Remote sensing Interstellar grains and meteorites Interstellar and cometary chemistry Origin of life Biogeochemical effects
Methods and capabilities for life in extreme environments	Methods to isolate and culture microbes Methods to study microbes Technology for non-contaminating sample recovery Sensors and sensing techniques Methods to study ancient microbial life Methods to study the potential for habitability

In concluding the first section of this review, we restate the importance of considering unique constraints surrounding life sciences in space: difficulty in distinguishing direct or indirect effects of microgravity, difficulty in identifying subtle effects, and expensive use of the space station. However, rapid advances in modern molecular biology and biotechnologies should facilitate identification of mechanisms underlying graviperception.

Research Opportunities for Space Life Sciences in the 21st Century

Space life sciences is one of the few research areas, besides nano-technology, which receives funding from three major agencies in the United States including NASA, NIH, and NSF. Research projects among those agencies are extremely diverse. They are also interdisciplinary and range from *molecular dynamics in nano-scale to a microbe evolution in interstellar space.* The program in NASA includes the study of diffusion-limited metabolic processes, while NIH supports research focusing on, for instance, a change in behavioral and cognitive ability of astronauts. NSF emphasizes life in extreme conditions as well as the origin of life. In this section of our review, we describe current research goals and scopes in the three U.S. federal agencies, and suggest projects for the 21st century.

NASA-sponsored research opportunities

The major goals of NASA's Life Sciences Division are to effectively use gravity and micro-gravity to enhance our understanding of fundamental biological processes, to develop the scientific and technological foundations for a safe and productive human presence in space, and to apply the knowledge and technology to improve quality of life on Earth. The six specific aims of the research announcement (NASA, 1999) were: (i) whether and how the development of cells, systems and organisms depends on gravitational force, (ii) the role of the genome and cellular structures in sensing and responding to gravitational force, (iii) how and for what purposes different organisms in the animal and plant kingdom sense and use gravity, (iv) how physical and chemical forces interact to determine biological structure and function, (v) the possible role of gravity in evolution, and (vi) the role of gravity in determining how the structure, function, and interactions of space and planetary ecosystems change over time. Possible research subjects corresponding to this NASA's announcement are summarized in Table 1.

NIH-sponsored research opportunities

The major objective of the NIH-sponsored research opportunities titled "Earth-based Research relevant to the Space Environment" is to stimulate basic, applied, and clinical biomedical and behavioral ground-based research that is relevant to human space flight or to advancing our understanding of the effects of the space environment on biological systems (NIH, 2000). Potential areas of research include neuroscience, musculoskeletal biology, immunology, cardiovascular functioning, integrative physiology, cognition and problem solving under stress and isolation, pharmacokinetics, drug metabolism and drug delivery, and the diagnosis and treatment of diseases or injury by both ground support and space flight crews. The specific research areas are summarized in Table 2.

NSF-sponsored research opportunities

NSF recently announced research opportunities titled "Life in Extreme Environments" (NSF, 1999). According to its program announcement, this interdisciplinary research program will explore the relationships between microbial organisms and the environments within which they exist, with a strong emphasis upon those life-supporting environments that exist near the extremes of planetary conditions. In addition, the program will explore planetary environments in our own solar system and beyond to help identify possible sites for life elsewhere. The program lists four themes such as microbial systems, extreme environments on Earth, planetary environments, and methods and capabilities for life in extreme environments (Table 3).

Concluding remarks

Gravity has been attracting challenging scientists and engineers to the interdisciplinary arena of life sciences in space. Gravity induces unique stimuli to living organisms. Recent advances in molecular and cellular biology revealed a network of stress-responsive signaling pathways such as mitogen-activated protein kinase pathways (Garrington and Johnson, 1999). The complete set of human genome data should facilitate identifying key regulatory elements in bone loss and muscle weakening of astronauts (Collins et al., 1998). Searching for microbes in space is something that has a potential to revolutionize our concept of life, and the life sciences. Answering the question as to how gravity contributes to unravel complexity of biological stress responses is a worthy endeavor in the 21st century.

Acknowledgements

We thank Profs. In-Ho Choi, Hak Ryul Kim, and Sang Dai Park (Korea) for organizing a special section on Gravitational Biology in KJBS.

References

Alpatov AM (2000) Molecular genetic approach to assess the effects of gravity on the circadian clock. *Space Utilization Res* 16: 23-26.

Asashima M, Kinoshita K, Ariizumi T, and Malacinski GM (1999) Role of activin and other peptide growth factors in body patterning in the early amphibian embryo. *Int Rev Cytol* 191: 1-52.

Bargmann CI (1998) Neurobiology of the *Caenorhabditis elegans* genome. *Science* 282: 2028-2033.

Berisio R, Vitagliano L, Sorrentino G, Carotenuto L, Piccolo C, Mazzarella L, and Zagari A (2000) Effects of microgravity on the crystal quality of a collagen-like polypeptide. *Acta Crystallpgr (Sec D)* 56: 55-61.

Blake JA, Richardson JE, Davisson MT, Eppig JT, and Mouse Genome Database Group (1999) *Nucleic Acids Res* 27: 95-98.

Boonstra J (1999) Growth factor-induced signal tranduction in adherent mammalian cells is sensitive to gravity. *FASEB J* 13 (Suppl): S35-S42.

Canizares CR (1998) A Strategy for Research in Space Biology and Medicine in the New Century. National Academy Press, Washington, DC.

Collins FS, Patrinos A, Jordan E, Chakravarti A, Gesteland R, Walters L, and Members of the DOE and NIH Planning Groups (1998) New goals for the U.S. Human Genome Project: 1998-2003. *Science* 282: 682-689.

Czeisler CA, Duffy JF, Shanahan TL, Brown EN, Mitchell JF, Rimmer DW, Ronda JM, Silva EJ, Allan JS, Emens JS, Dijk DJ, and Kronauer RE (1999) Stability, percision, and near-24-hour period of the human circadian pacemaker. *Science* 284: 2177-2181.

Garrington TP and Johnson GL (1999) Organization and regulation of mitogen-activated protein kinase signaling pathways. *Current Opinion Biol* 11: 211-218.

Holick MF (1998) Perspective on the impact of weightlessness on calcium and bone metabolism. *Bone* 22 (Suppl): 105S-111S.

Ikenaga M, Yoshikawa I, Kojo M, Ayaki T, Ryo H, Ishizaki K, Kato T, Yamamoto H, and Hara R (1998) Mutations induced in *Drosophila* during spaceflight. *Biol Sci Space* 11: 346-350.

Ingber, D (1999) How cells (might) sense microgravity. *FASEB J* 13 (Suppl): S3-S15.

Kiefer J and Pross HD (1999) Space radiation effects and microgravity. *Mutation Res* 430: 299-305.

Klaus DM (1998) Microgravity and its implication for fermentation biotechnology. *Trends Biotechnol* 16: 369-373.

Lawler A (2000) Space Station Biology Panel says NASA must show results fast. *Science* 287: 1728-1729.

Littke W and John C (1986) Protein single crystal growth under microgravity. *J Crystal Growth* 76: 663-672.

Mogami Y, Koike H, Yamashita M, Izumi-Kurotani A, and Asashima M (1996) Early embryogenesis of amphibians in space: AstroNewt for the space embryology in IML-2 and SFU. *Adv Astronau Sci* 91: 1089-1097.

Moore RY (1999) A clock for the ages. *Science* 284: 2102-2103.

Musgrave ME, Kuang A, and Matthews SW (1997) Plant reproduction during spaceflight: importance of the gaseous environment. *Planta* 203: S177-S184.

NASA Research Announcement NRA 99-HEDS-02 (1999) Research opportunities in space life sciences: gravitational biology and ecology.

Neff AW, Yokota H, Chung HM, Wakahara M, and Malacinski GM (1993) Early amphibian (anuran) morphogenesis is sensitive to novel gravitational fields. *Dev Biol* 155: 270-274.

NIH Program Announcement PA-00-088 (2000) Earth-based research relevant to the space environment.

NSF Program Announcement NSF 00-37 (1999) Life in extreme environments (LExEn).

Reichhardt T, Abbott A, and Saegusa A (1998) Science struggles to gain respect on the space station. *Nature* 391: 732-737.

Sanchez C, Lachaize C, Janody F, Bellon B, Roder L, Euzenat J, Rechenmann F, and Jacq B (1999) Grasping at molecular interactions and genetic networks in *Drosophila melanogaster* using FlyNets, an internet database. *Nucleic Acids Res* 27: 89-94.

Schmitt D (1999) Workshop conclusions and recommendations (ESA/NASA Workshop on Cell and Molecular Biology Research in Space, Belgium, 1998). *FASEB J* 13 (Suppl): S175-S177.

Souza KA, Black SD, and Wassersug RJ (1995) Amphibian development in the virtual absence of gravity. *Proc Natl Acad Sci USA* 92: 1975-1978.

Spangenberg DB, Lattanzio FA Jr, Philpub C, Schwarte R, Coccaro E, Lowe B, and Philput J (1997) Effects of weightlessness on budding and ephyra development in *Aurelia aurita* (Linnaeus, 1758) (Scyphozoa: Semaeostomeae). *Proc 6th Int Conf Coelenterate Biol* 1995: 447-453.

Suzuki S (1998) A theoretical model for simultaneous mixed irradiation with multiple types of radiation. *J Radiat Res* 39: 215-221.

Tei H, Okumura H, Shigeyoshi Y, Fukuhara C, Ozawa R, Hirose M, and Sakaki Y (1997) Circadian oscillation of a mammalian homologue of the *Drosophila* period gene. *Nature* 389: 512-516.

Yokota H, Ikeuchi M, Neff AW, and Malacinski GM (1998). Frontiers in biology: NanoBiotech lab for the space station. *J Jpn Soc Microgravity App* 15 (Suppl II): 598-601.

[Received June 23, 2000; accepted July 16, 2000]