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Genes in Space Finalists: Contributing Ideas to Advance Space Biology

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INTRODUCTION

Fifty years ago, humankind took its first steps on the moon, inaugurating a tradition of space travel that many hope will take us to Mars and beyond. In the years since the moon landing, humans have established a permanent presence in space via the International Space Station (ISS). Continuously staffed by a crew of astronauts, the ISS serves as a hub for scientific research of all kinds: from materials science to tissue engineering and even agriculture.

Aspiring space explorers need not wait to get involved in research on the ISS. In 2015, the Genes in Space program was established to grant students access to this extraordinary resource. Genes in Space is a competition for middle and high school students who are interested in becoming space biology pioneers. Contestants submit proposals for DNA science experiments that would expand our understanding of how the conditions of space – e.g., microgravity and cosmic radiation – affect our understanding of biology and specifically genetics. A team of scientists reviews the proposals and identifies five finalist teams that are then paired with mentors to further develop their proposals into detailed scientific plans. From these finalists, a panel of judges including scientists, engineers, and educators selects one winner whose experiment is flown to the ISS and carried out by astronauts. Their results inform our nascent understanding of how life is affected by cosmic conditions and will ultimately aid in the development of safeguards against the risks of spaceflight.

In the years since Genes in Space was founded, more than 5,000 students have risen to the challenge by submitting a proposal, and a total of six student experiments have flown to the ISS. Past winners have been responsible for some significant steps forward for space biology: they've studied the impact of space travel on processes ranging from immune function to chromosome stability to aging. Beyond the contest winners, all Genes in Space participants contribute to the advancement of molecular biology in space by sharing their ideas with the scientific community. Here, the 2019 Genes in Space finalists are publishing their proposals in hopes that the creativity and careful thought they put into this work will inspire the next generation of explorers and innovators.

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Genes in Space

Fruit Salad in Space: A Single-cell Approach to Understanding the Molecular Mechanism of Bone Density Loss in Space

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ABSTRACT

Bone remodeling is a dynamic process of formation and resorption that involves many different cell types, including osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells). Maintaining healthy bone density requires a balance between formation and resorption. If resorption outpaces formation, bone density decreases. Bone density loss is a major problem for space travel. Astronauts lose about 1% of their bone mass per month in space, may never fully recover this density, and have an increased risk of fractures [1]. We propose using single-cell analysis to discover the molecular mechanisms of bone density loss in space. This understanding would help us develop methods to prevent bone density loss in space, improving astronaut health and removing one barrier to human exploration of deep space.

Prior analyses have shown that expression levels of key genes in bone formation change in space [2]. However, all previous work used bulk-cell analysis, where the gene expression from all cells in a population are mixed together like in a fruit smoothie. Alternatively, single-cell analysis gathers data from each cell individually, where the characteristics of each part are visible like in a fruit salad. Thus, single-cell analysis allows us to understand whether overall changes in gene expression occur across all cell types, in specific cell types, or in the proportion of cell types expressing the gene. This provides a comprehensive data set from which we can derive the biological mechanisms driving bone density loss. Single-cell has been very successful on Earth but has never been performed in space [2], so another objective is to show that this method can be effectively performed off our planet. A successful proof of concept would open the door to many future space-based single-cell analyses.

We propose constructing bone organoids on Earth from mouse embryonic stem cells [3]. Organoids are 3D tissue models grown in vitro; they capture the three-dimensional structure of bones that is essential to their ability to function [4]. Once grown to maturity on Earth, half will travel to the ISS while the other half stay on Earth as a control. scRNA-seq will be performed on all samples at multiple time points to study the differentiation processes of the cells and the effects of space exposure on bones. The first time point will be immediately after arrival at the ISS to determine how macrogravity during launch impacts the organoids.

The expression level of each gene for each cell will be measured, which will then allow for the cells' gene expression profiles to be clustered. Each cluster represents a different cell type, allowing us to separate them and obtain the expression profile of each. Based on the expression profile of each cell type, we will investigate several important aspects of the molecular mechanisms of bone density loss in space: 1) how the ratio of osteoblasts to osteoclasts in the space samples differs from the Earth controls, 2) how space changes expression levels of genes that regulate bone cell differentiation, and 3) how the differentiation processes of osteoblasts and osteoclasts change in space. The biological insights from this experiment will greatly aid scientists in developing proper countermeasures for bone density loss during space travel. The single-cell analysis established in this experiment will also pave the way for understanding other biological issues of space exploration.

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Genes in Space

Sustaining the Human Race Beyond the Earth: Studying The Space-Induced Changes to Gametogenesis

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ABSTRACT

In recent years, the interest in long-range space travel and establishing extraterrestrial colonies has grown tremendously, moving the topic from the realm of science fiction to real-life scientific research. To allow for long-range space travel, many problems have to be addressed. Key among them is understanding the challenges facing reproduction in the space environment to ensure multigenerational growth and survival of any human that undertakes such space travel.

Gametogenesis, the process whereby mature sperm and eggs are produced, is believed to be negatively impacted by the space environment, resulting in increased germline mutations, infertility, and embryo mortality [1]. However, the underlying mechanisms that cause defects in gametogenesis under microgravity are not well understood. Studies of cell morphology and structure in space have shown disorganization of the fundamental structural component of the cell: the cytoskeleton. The cytoskeleton gives the cell its form and is important for cells to fulfill their functions. Moreover, cytoskeletal genes are also downregulated in cells in space [2].

Since gametogenesis requires proper cytoskeletal function for chromosome separation and cell division, cytoskeletal disruptions caused by the space environment may affect gametogenesis significantly. I propose to investigate the effect of spaceflight on the cytoskeleton in germ cells, as I hypothesize that spaceflight-induced cytoskeletal destabilization will disrupt germ cell maturation. To address the disruption of the cytoskeleton during space flight, the drug melatonin will be used to treat germ cells in space, as melatonin has been shown to promote cytoskeletal stability [3]. To test this hypothesis, we will study spermatogenesis, the formation of sperm cells, using the fruit fly Drosophila melanogaster as our model system. Drosophila melanogaster is a well-established model of spermatogenesis and is convenient for use on the space station. This experiment will utilize two types of fruit flies: a Vasa-Gal4;UAS-laminGFP and Fuca-Gal4;UAS-laminGFP transgenic fly which will produce GFP in the nuclear membrane of maturing germ cells allowing these cells to be isolated through immunopurification. This can be achieved by using the magnetic bead pulldown method with an anti-GFP antibody. The other type, βtub85D mutant fly, experiences cytoskeletal instability and will, therefore, be more sensitive to the cytoskeletal destabilization effects of the space environment. Each of the two types will be divided into two subgroups. One will be untreated, and one will receive 4 mM melatonin orally through food [4]. The effect of the space environment and whether melatonin could be used to prevent negative effects will then be measured through the following methods:

1) Measure the changes in sperm-development and cytoskeleton function using qPCR of specific spermatogenesis stage marker and cytoskeletal genes and RNA sequencing.

2) Conduct a fertility assay by assessing whether space travel impacts the number of eggs laid and the number of viable offspring produced by the flies.

3) Observe the cell shape and structure of the cytoskeleton. A sample from each fly group will be fixed and sent back to earth for antibody staining (e.g. actin, tubulin) and observation through microscopy.

Through these experiments, we will gain knowledge regarding how the space environment may impact reproductive capabilities and whether melatonin can be used as a simple way to mitigate some of the negative effects of space travel on the development of germ cells. In the long term, these findings will serve as an important contribution to enable mankind to further long-range space travel and future space endeavors into new and fruitful territories.

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Assessing the Role of Viral Reassortment in Increased Viral Outbreaks on the International Space Station

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ABSTRACT

Astronauts on the International Space Station (ISS) are more prone to viral outbreaks, which can lead to life-threatening infections in space with limited medical resources [1]. Understanding the mechanisms that influence virulence in microgravity conditions is important when considering life in space, although this topic remains understudied. Influenza A (IAV) is a potent virus that infects millions of people yearly. One reason why IAV is problematic is that it mutates quickly through a process called viral reassortment. Viral reassortment happens when multiple viruses infect the same cell and viral gene segments are exchanged during the packaging stage of the virus life cycle [2]. In severe cases, humans don't have antibodies to recognize reassorted viruses, causing pandemic level strains such as the H1N1 Spanish Flu and the pH1N1 Swine Flu [3,4]. On Earth, the ability to reassort provides genetic diversity and can increase virulence for a virus; however, the effect of reassortment on virulence under microgravity conditions has not been studied. In the following study, we propose the first experiments to investigate how viruses, specifically IAV, may reassort uniquely in microgravity.

Based off previous research showing microgravity weakens immune systems and impairs cellular function, we're predicting that viral reassortment rate will increase under microgravity conditions. We propose to evaluate this hypothesis by observing three factors of reassortment that may change under microgravity: segment mismatch, time between coinfection, and density of virus particles [5]. The first factor, segment mismatch, occurs when epistatic signals between packaging RNA segments restrict the successful genome combinations of reassorted viral progeny. Microgravity has been shown to alter this type of signaling within cells [6], and similar mechanisms could disrupt segment mismatch signaling. The second and third factors, time of coinfection and density of virus particles, may be altered by a weakened host immune system under microgravity conditions, leading to altered virulence.

To evaluate reassortment on the ISS, we plan to first use the Tomato Spotted Wilt Virus (TSWV), a virus that is benign in humans and thus poses little risk to the astronauts aboard the ISS. Both the viruses, TSWV and IAV, are segmented, negative stranded, and single-part, showing close alignment in the features pertaining to reassortment. We will co-infect a young *N. tabacum* plant with two different strains of TSWV: TSWV-D and TSWV-10. These strains have identifiable genetic markers which allows us to identify the reassorted progeny. The co-infected plant will be left on the ISS in order for viral strains to replicate and reassort within the plant. A plaque assay will allow us to isolate single viral particles and extract RNA for sequencing via RT-PCR and Nanopore MinION sequencer. Gel electrophoresis of PCR products and sequencing results will indicate the reassorted viruses. Additionally, we will test IAV and TSWV in artificial microgravity on Earth to confirm that TSWV is an accurate model.

This study will grant insight into viral mechanisms in microgravity and help take a step forward in saving lives, both on Earth and in space.

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Oxidative Stress and Base Excision Repair in Space May Threaten Long-Term Astronaut Health

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ABSTRACT

In space, increased oxidative stress and impaired nuclear and mitochondrial DNA repair mechanisms are likely to place astronaut health at risk during long-duration missions. Free radicals and reactive oxygen species (ROS)/reactive nitrogen species (RNS) play a central role in the redox biology of the human body and are crucial for cellular functions [1]. A balance of these radicals is necessary for maintaining normal physiological conditions, as an accumulation of free radicals causes DNA damage in a condition known as oxidative stress [2]. Microgravity and increased radiation exposure in space might cause astronauts to be more susceptible to oxidative DNA damage, as cells more readily generate free radicals [3]. Compounding these risks, base excision repair (BER), the primary mechanism for repairing oxidative damage, might be impaired in microgravity. Unrepaired DNA damage in the nucleus can cause cancer, while defective BER in mitochondria is associated with the development of neurodegenerative disorders, given the critical role of mitochondria in cellular energy production and programmed cell death pathways [4,5].

The genomic integrity and cellular defense mechanisms of astronauts are of particular concern, as previous studies have indicated heightened oxidative DNA damage as well as downregulated DNA repair gene expression in simulated microgravity [6]. Additionally, BER deficiency has been observed in high-stress conditions such as those found in space [7]. Based on these observations, we propose to investigate BER to evaluate whether extended exposure to the space environment might pose previously-unanticipated health risks to astronauts [8].

We have designed several approaches to assess BER efficiency in microgravity. First, a biotin-tagged hairpin DNA fragment with a pre-damaged base on one strand will be transfected into mitochondria or nuclei in space or on Earth. After incubation, streptavidin retrieval will extract the hairpin DNA. Repair efficiency will be assayed by a PCR amplification that only amplifies the repaired product, using amplification of the other strand as a housekeeping control. Analysis of the two sub-pathways of BER, short-patch and long-patch repair, is necessary to pinpoint the exact mechanism that may be impacted. Short patch and long patch BER are two distinct enzymatic sub-pathways in which specific oxidative damages are removed, depending on the structural and chemical properties of the lesion. The efficiency of both will be investigated separately using two different fluorescent-tagged DNA templates designed specifically to analyze either short-patch or long-patch repair. We will treat each template with lysates from nuclei or mitochondria from cells grown in space or on Earth and analyze changes in the length of the template to evaluate successful repair by gel electrophoresis.

BER deficiency might be caused by multiple cellular mechanisms, including reduced gene expression, aberrant transcript splicing, or aberrant protein localization. RNA-seq of cells in microgravity and on Earth will evaluate whether BER genes are expressed and spliced appropriately in space, while Western Blot of nuclear and mitochondrial lysates will determine whether protein localization is impaired. These investigations will inform future studies regarding potential therapeutic targets that might mitigate health risks due to impaired BER. Because the space environment might lead to heightened oxidative damage, the defense and repair capacity of human cells in space must be evaluated. Cognitive disabilities and cancers associated with BER deficiency would be detrimental to long-duration space exploration, thus motivating the investigation of these and similar cellular defense mechanisms against oxidative stress.

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