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# Diacylglycerol kinase is downregulated in the Drosophila Seizure Mutant during Spaceflight

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**Abstract**

***Accelerated aging in space is detrimental to long-term space missions. The environmental conditions in space (e.g., microgravity and radiation) cause harmful effects similar to those seen during aging. As the mechanistic pathways underlying accelerated aging in spaceflight are not fully understood, the identification of critical targets for promoting longevity in spaceflight remains challenging. We analyzed genomics data from the GLDS-207 project to identify potential targets related to longevity. Analysis of RNA-seq data from four Drosophila variants using the GeneLab Galaxy platform indicated that spaceflight significantly affected differential gene expression in the heads of flies, specifically in the seizure (sei) mutant, which alters the voltage gated potassium channels in the cell membrane. Spaceflight induced a significant decrease in the expression of the retinal degeneration A gene (rdgA) in mutant flies that survived the 30-day space mission. This gene encodes for the protein diacylglycerol kinase (DGK), which modulates the activation of the mechanistic target of the rapamycin (mTOR) signaling pathway, known to negatively regulate aging. Therefore, DGK may be a potential target for promoting longevity in space conditions. Further investigation of the effects of decreased rdgA expression on the lifespan of other organisms under spaceflight conditions will clarify the role of DGK in promoting longevity.***

**Keywords**

*Diacylglycerol kinase • Longevity • Spaceflight • Drosophila • GeneLab Galaxy.*

## Introduction

Organisms experience accelerated aging in space, exemplified by health effects including bone loss, muscle atrophy, and decreased cardiovascular functional capacity, among others (Verkinos and Schneider, 2010; Kandarpa, 2019; Tavassoli, 1986). As NASA shifts its focus towards manned missions beyond Low Earth Orbit (LEO), studies concerning the longer-term health effects of spaceflight exposure outside of the Earth's magnetic field are necessary for preserving human health during long-duration spaceflight. Research conducted in the space environment provides a unique perspective that is critical for understanding human health conditions, including aging. As aging is characterized by the deterioration of physiological and biochemical functions in different tissues of organisms (López-Otín et al., 2013), the analysis of omics data collected from model organisms subjected to spaceflight conditions may provide important insights on potential targets for promoting longevity.

In this study, a computational/bioinformatics analysis of omics data from GeneLab Data System's GLDS-207 project (Correlated Gene and Protein Expression in Heads from *Drosophila* Reared in Microgravity) was conducted with the goal of identifying critical targets for promoting longevity. *Drosophila* is an ideal organism for spaceflight research because of its cost-effectiveness and relative simplicity when compared to the base costs for sustaining more complex organisms beyond LEO experiments (Tolwinski et al., 2017). As the average lifespan of *Drosophila* is 50-70 days, they are also a good model for studying aging. The data from the head tissue was made available through the Biospecimen Sharing Program, as the original study was focused on cardiac tissue and cardiac disease implications in spaceflight (Walls et al., 2020). Analyses of this data set identified a potential target for promoting longevity, which could be critical for long-term space missions.

## Materials and Methods

### GLDS-207 study methodology

Data analyzed in this study was generated by the GLDS-207 project: Correlated Gene and Protein Expression in heads from Drosophila reared in microgravity. In the GLDS-207 study, four different *Drosophila* fly lines were used: the Canton S laboratory wildtype, *sei* mutant, KCNQ180 mutant, and genetic control KCNQ97. The flies were kept in the vented fly box (VFB) for a 30-day mission in space (Walls et al., 2020),

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then returned live, and the head tissue was dissected and processed on Earth. Each of the 15 vials contained eight female and five male adult *Drosophila* flies. Ground control samples were prepared and maintained under identical conditions as the flight experiment counterparts. The metadata from this study included the omics analysis of RNA and proteins extracted from the samples (heads of microgravity-reared *Drosophila*).

**Experimental groups and analytical design**

In this study, RNAseq data from the *sei* mutant and the wild-type Canton S flies were analyzed. Four separate analyses were run, comparing the spaceflight and ground control samples for the *sei* mutant data, the spaceflight and ground control samples for the Canton S wildtype data, the ground controls of both fly lines, and the spaceflight samples of both fly lines. This comparative analysis allowed us to determine what conditions are most affecting differential gene expression.

**Computational analyses tools**

GeneLab Galaxy, a publicly available computational analysis platform that includes utilities for library preparation, normalization, bias removals (caused by sequencing errors), and quantification of differential gene expression was used (Beheshti et al., 2019). Then, the data was visualized to identify genes of interest using the following sequencing tools: FastQC, TrimGalore!, RNA STAR, featureCounts, MultiQC, DESeq2, Volcano Plot Visualization, goseq, and fgsea.

## Results

First, the data was analyzed to assess the statistical significance. Analysis of DESeq2 outputs comparing the spaceflight and ground control samples for the *sei* mutants revealed that most differential expressed genes exhibited a p-value < 0.05. The bar graph (Figure 1) shows the p-value distribution for the *sei* mutant flight versus ground control groups. As the observed changes for most of the differentially expressed genes are significant, the histogram suggests that these changes could be attributed to the effect of space on the *sei* mutants.

The principal component analysis (PCA) plot was generated as a two-dimensional visualization of the relative correlation in gene expression within specific test groups. The variance between the flight and ground control samples was found to be greater in the *sei* mutant fly line than in the wildtype. This indicates the increased differential expression of genes in the mutants. Moreover, the variance (72%) in the PCA reveals that the principal impact on the samples is spaceflight (Figure 2).

A volcano plot was created to characterize this differential gene expression and visualize the most differentially expressed genes (Figure 3). The volcano plot indicated that *rdgA, retinal degeneration A,* was significantly downregulated. Moreover, this gene has been reported to influence the aging process in other studies (Lin et al., 2014). Down-regulation of this gene in a *Drosophila* mutant strain during spaceflight may imply its potential role in influencing longevity and altering lifespan during long-term space missions.

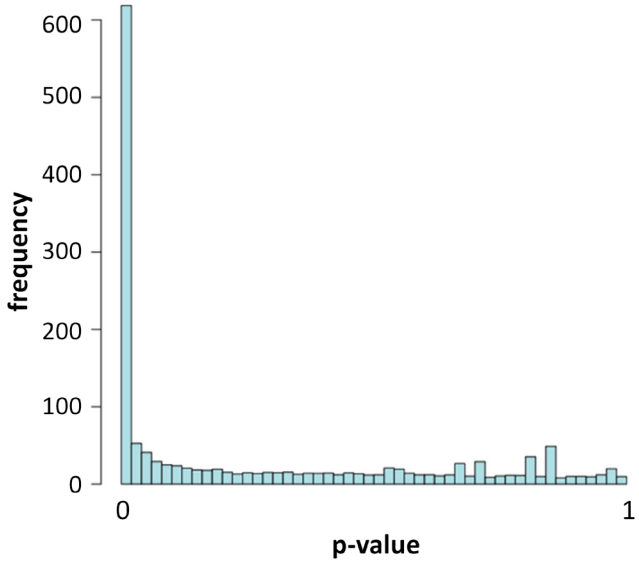


**Figure 1.** Statistical significance analysis. This histogram represents the statistical significance of the data based on p-value, specifically for the *sei* mutant flight and ground control comparison. If the p-value is less than 0.05, the data is significant. As the peak is closest to the origin, this graph indicates that this is an excellent data set for further analyses.

GoSeq pathway analysis was used to identify biological processes and molecular functions that were impacted by differential gene expression. Proteolysis and serine-type endopeptidase activity were found to be highly enriched (Figure 4).

## Discussion

The results of this study indicate that in spaceflight conditions *rdgA* expression in the *sei* mutant is decreased. The *rdgA* gene encodes for DGK, an activator and regulator of the mechanistic target of the rapamycin (mTOR) pathway (Torres-Ayuso et al., 2015). The mTOR pathway is a serine/threonine pathway and has been associated with processes of aging through multiple mechanisms (Papadopoli et al., 2019). Studies have shown that a knockdown of the *rdgA* gene led to a significant increase in lifespan of about 44% (Lin et al. 2014). Knockdown of DGK extends lifespan and enhances response to oxidative stress.

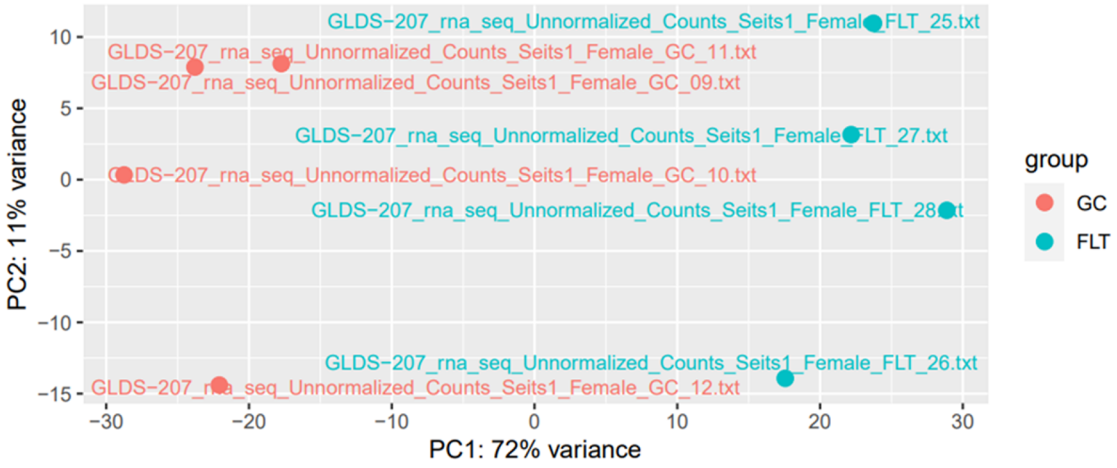


**Figure 2.** The principal component analysis. PCA plot signifies the variance between the spaceflight and ground control groups. More variance in PC1 indicates a higher difference in the pattern of expression between ground controls and flight samples. This pattern is observed in the *sei* mutant flight and ground control sample comparisons.

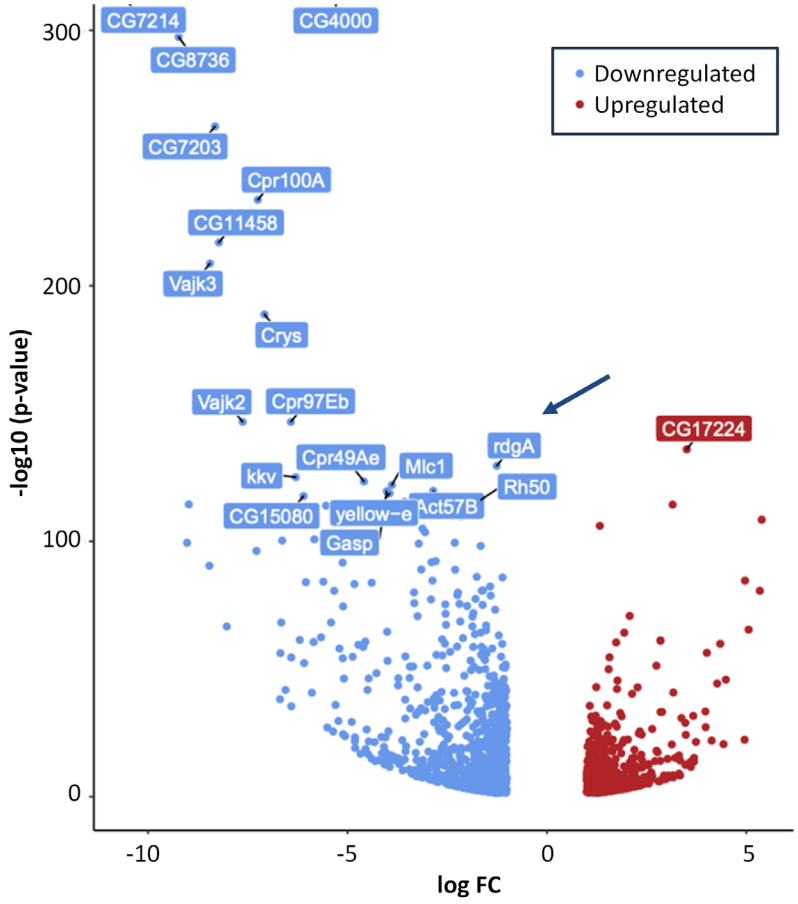


**Figure 3.** Identification of gene of interest. The volcano plot generated after DESeq 2 analysis for the *sei* mutant ground control and flight sample comparison is illustrated. The top twenty most significantly up or down-regulated genes are labeled on the plot. Note that rdgA, our gene of interest, is significantly downregulated.

| Gene Ontology Analysis | |
|---|---|
| **Biological Process** | Proteolysis |
| | Transmembrane Transport |
| | Oxidation-Reduction Process |
| **Molecular Function** | Serine-Type Endopeptidase Activity |
| | Zinc Ion Binding |
| **Cellular Component** | Extracellular Space |
| | Extracellular Region |
| | Integral To Membrane |

**Figure 4.** Gene Ontology Analysis. GoSeq pathway analysis indicated that proteolysis was a highly enriched biological process and serine-type endopeptidase activity was a highly enriched molecular function.

Spaceflight induces oxidative stress due to an imbalance of reactive oxygen species (ROS) in cells and tissues (Stein, 2002). Oxidative stress has been shown to affect the voltage gated potassium channels in *sei* mutants (Liu et al., 2002). Although *sei* mutants exhibited higher resistance to the toxic effects of hydrogen peroxide relative to wild type controls, the underlying mechanism is not known (Hill et al., 2019). Since both spaceflight environment and hydrogen peroxide exposure induce oxidative stress and spaceflight conditions decreased the *rdgA* expression in *sei* mutants that survived the spaceflight, it is likely that the decrease in *rdgA* contributed to its resilience. Moreover, DGK, the enzyme coded by rdgA, has been shown to regulate oxidative stress and aging by inversely modulating TOR signaling. So, the negative effects of oxidative stress, which contributes to the aging of organisms, are potentially reduced by the changes in the expression of the *rdgA* gene in the *sei* mutant.

Activation of the mTOR pathway causes excessive protein synthesis, inhibits autophagy, dysregulates mitochondria, and results in accelerated senescence (Chen et al., 2021). As DGK-derived phosphatidic acid acts as a mediator of mTOR signaling (Avila-Flores et al., 2005), the expression level of DGK determines whether the pathway is activated or not. Moreover, DGK inhibition also enhances an organism's protection against oxidative stress (Lin et al., 2014), which is also a major contributor to accelerated aging. Therefore, DGK appears to be a promising target as it directly impacts two critical pathways that lead to accelerated aging. Follow-up studies in higher organisms may lead to new insights and clarify the mechanism by which DGK downregulation could combat accelerated aging in space and on Earth.

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## Author Disclosure Statement

No competing financial interests exist.

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