Is spaceflight as consequential as primary mitochondrial disease in sleeplessness?

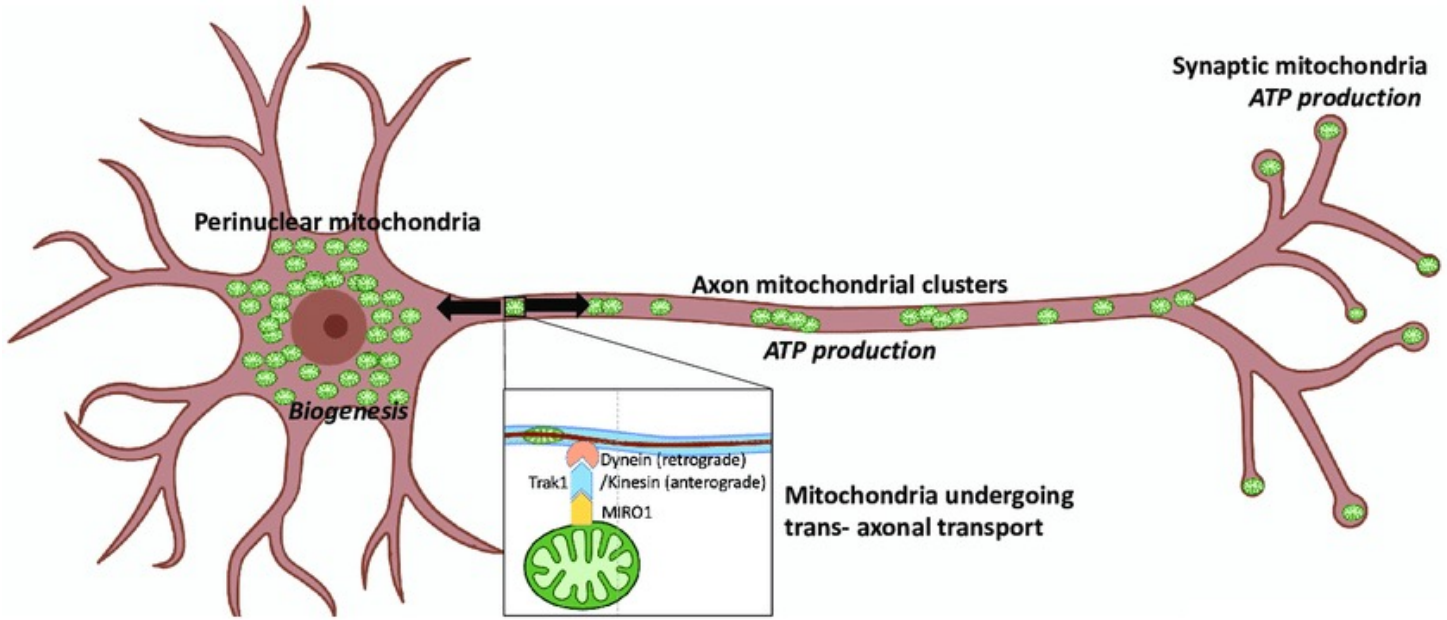
Viktor Stolc, PhD

NASA Ames Research Center

Hypersomnolence/Hypersomnia Meeting

Mitochondria in Neurons and Other Cells of the CNS

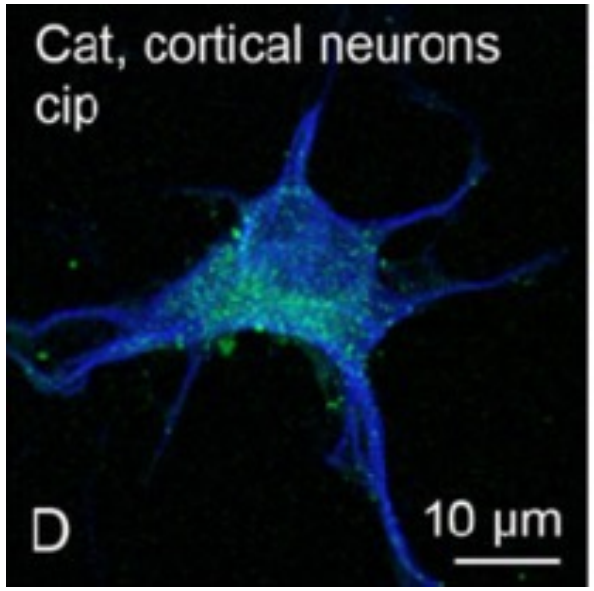
Image:
Creative Commons
By 4.0

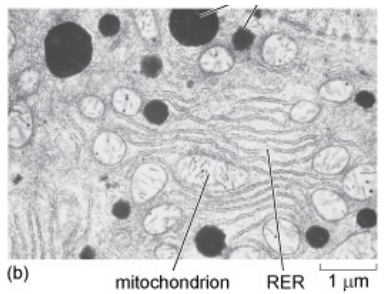


- Neurons are jam-packed with mitochondria.
- Mitochondria are clustered around the nucleus of the cell body.
- Mitochondria are also present along axons and at synapses.
- Neurons have developed an efficient method of transporting mitochondria to the synapses, to meet the high energy demands of synaptic signaling.
- Neuronal cell energetics facilitated by mitochondria plays a strong role in neural development, synaptogenesis and neuronal apoptosis.
- Other cell types in the brain, such as oligodendrocytes, microglia and endothelial cells are rich in mitochondria too.

Peroxisomes in the CNS

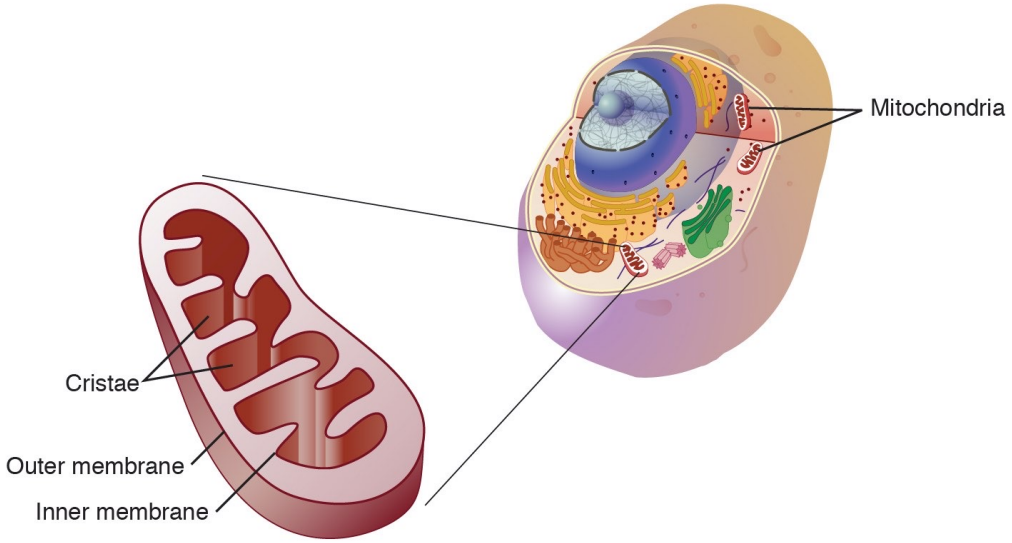
Image Credit:
NIH





- Neurons are jam-packed with peroxisomes.
- These tiny organelles (smaller than mitochondria) are present in oligodendrocytes, microglia and endothelial cells of the brain, too.
- Peroxisomes play an important role in metabolism in the CNS, and elsewhere in the body.
- Peroxisomes contain enzymes that break down fatty acids and amino acids, resulting in the generation of hydrogen peroxide.
- Peroxisomes also contain catalase, which breaks down hydrogen peroxide, which prevents hydrogen peroxide damage to cells.

What's So Special About Mitochondria?



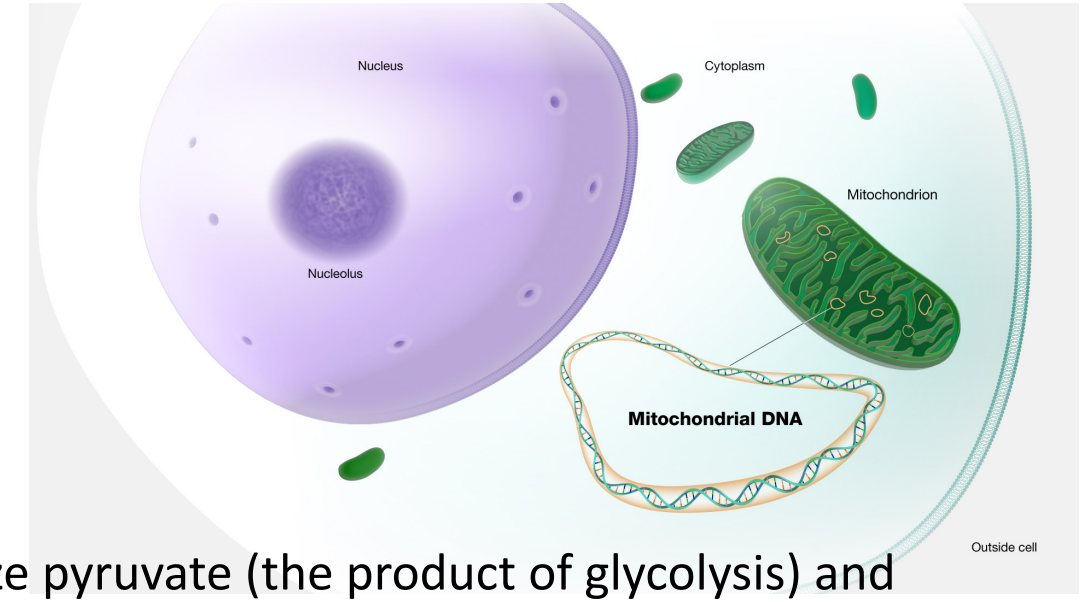


Image: Creative Commons by 4.0

- The main function of mitochondria is to metabolize pyruvate (the product of glycolysis) and generate ATP (*via* the Kreb's cycle, i.e., "cellular respiration").
- All cellular processes that rely on ATP, therefore, rely on mitochondria.
- Mitochondria contain their own DNA—separate from the DNA in the nucleus of the cell.
- The proteins present inside mitochondria are encoded by the mitochondrial DNA—for the most part.
- Some proteins found inside mitochondria are actually encoded by nuclear DNA. They get transcribed and translated outside of the mitochondria and then get transported into the interior of the mitochondria. **Reactive Oxygen Species** serve a "time-keeping" function as switches of various cellular processes and physiological rhythms, including sleep.

What About Primary Mitochondrial Disease?

- Mutations in mitochondrial DNA, or mutations in nuclear DNA that encode mitochondrial proteins can give rise to a wide ranges of “Primary Mitochondrial Diseases.”
- Most of these Mitochondrial Diseases, not surprisingly, affect cellular energetics.
- Lactic acidosis is seen commonly (because of defects in the enzyme pyruvate dehydrogenase).
- Neurological and neuromuscular effects are common.

Kearns-Sayre syndrome (KSS)

- First multisystem mitochondrial disorder to be defined clinically
- Large-scale mitochondrial DNA deletions
- Onset in youth
- Retinitis pigmentosa, external ophthalmoplegia, complete heart block,
- ± lactic acidosis, among many other signs and symptoms, depending on the exact pattern of mtDNA deletions.
- Diabetes is common.

- Neurological Manifestations:
  - --Ataxia
  - --Neuropathy
  - --Impaired cognition, early dementia

Progressive External Ophthalmoplegia (PEO)

- Like KSS, PEO is caused by deletion mutations in mitochondrial DNA.
- Can also be caused by mutations in mitochondria-relevant nuclear genes.
- Onset of symptoms age 18-20.
- Ptosis (eyelid drooping) usually the first sign.

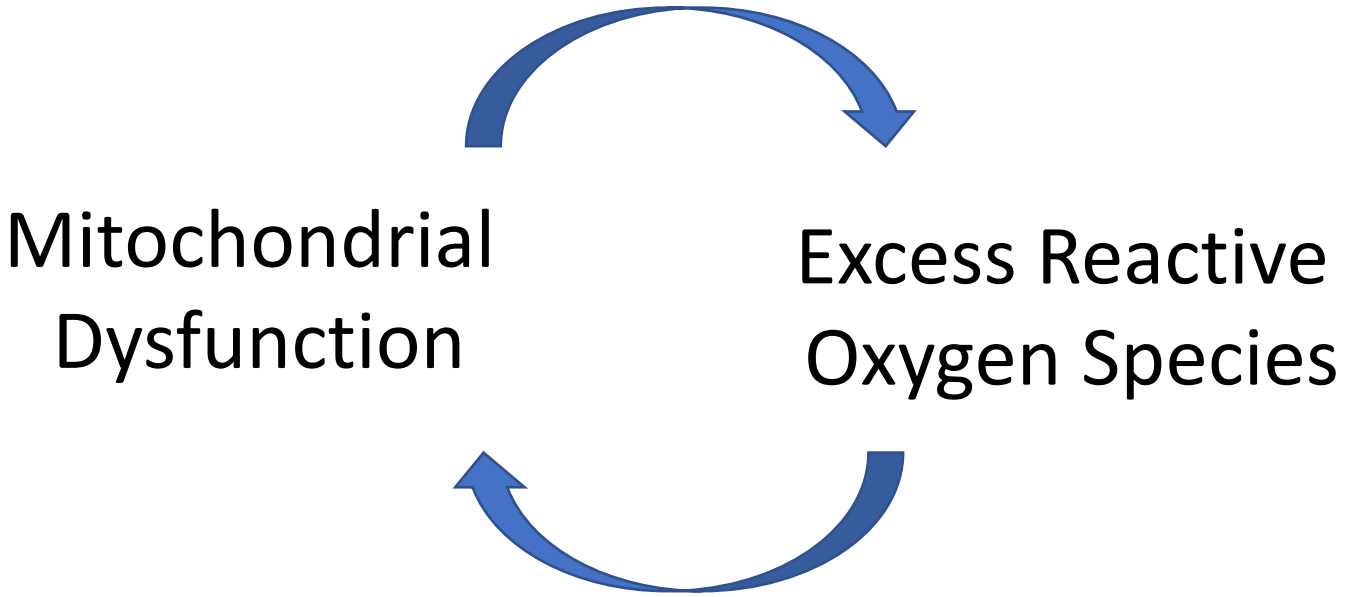
- Neurological Symptoms
  --Central Sleep Apnea

PDC Deficiency (Pyruvate Dehydrogenase Complex Deficiency)

- Most are X-linked nuclear DNA-encoded
- Onset of symptoms in childhood
- Characterized by hypotonia, psychomotor retardation, seizures, and lactic acidosis.

- Neurological Symptoms
  --Deficient autonomic central control of ventilation (Central Hypoventilation Syndrome)
  --Global autonomic dysfunction

The Yin and Yang of Mitochondrial Dysfunction and Reactive Oxygen Species



Metabolic dysfunction significantly impacts neurological function, including sleep disorders and other manifestations:

•**Insomnia**: Metabolic disorders like obesity, diabetes, and metabolic syndrome heighten the risk of insomnia due to hormonal imbalances, inflammation, and glucose metabolism disruptions.

•**Excessive Daytime Sleepiness**: Conditions such as sleep apnea and narcolepsy are often associated with metabolic dysfunction, particularly obesity, exacerbating fragmented sleep and daytime sleepiness.

•**Restless Legs Syndrome (RLS)**: Metabolic conditions like iron deficiency anemia and renal failure worsen RLS symptoms, leading to sleep disruptions.

•**REM Sleep Behavior Disorder (RBD)**: RBD, characterized by dream enactment during REM sleep, is linked to neurological conditions like Parkinson's disease involving metabolic dysregulation.

•**Cognitive Impairment and Neurodegenerative Diseases**: Insulin resistance and glucose dysregulation contribute to neurodegenerative diseases like Alzheimer's, often accompanied by sleep disturbances exacerbating cognitive decline.

•**Mood Disorders**: Metabolic dysfunction contributes to mood disorders such as depression and anxiety, intertwining with sleep disturbances via neurotransmitter dysregulation, inflammation, and neuroendocrine alterations.

What is Metabolic Dysfunction at the Cellular Level in the CNS?

Excessive Reactive Oxygen Species (ROS) induce mitochondrial dysfunction, impacting the nervous system, notably sleep. Mitochondrial diseases can directly influence sleep, leading to excessive daytime sleepiness due to impaired energy production, sleep-disordered breathing from weakened respiratory muscles, and fragmented sleep with daytime fatigue due to overall cellular dysfunction.

Excess ROS also trigger Peroxisomal dysfunction, causing the buildup of toxic substances damaging neurons. Associated sleep disorders, such as central sleep apnea and insomnia, are less understood but may involve dysfunction in breathing control regions and disrupted cellular processes regulating sleep.

Examples how metabolic stress and excess ROS impact cells through various pathways:

1. *Heat* exposure directly leads to protein degradation and DNA damage, potentially resulting in genetic mutations, cell dysfunction, or death.

2. *Heat or noise* triggers cortisol induction as a response, which may contribute to genetic mutations.

3. Exposure to metabolites like acetic acid or salt can induce damage and degradation to proteins and DNA, potentially leading to processes such as apoptosis.

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Questions:

Might systemically elevated ROS contribute to brain disease in the same way that ROS generated locally in the brain can cause CNS pathology?

Can ambient noise induce excess ROS in the brain?

Pathophysiology underlying increased Reactive Oxygen Species (ROS)

Clinically recognized *subcellular* pathologies associated with metabolic dysfunction:

1. Oxidative stress (excess reactive oxygen species, ROS),
2. Mitochondrial dysfunction,
3. Inflammation,
4. Epigenetics (Methylation),
5. **DNA mutation (GWAS)**

What do these cellular dysfunctions have in common?

Excess ROS!

Sleep in space: the role of ROS?

- Sleep is critical for human health and is required by all mammals.
- Timing and duration of sleep are controlled by the circadian system, which keeps a 24-h internal rhythm that synchronizes to environmental stimuli. All organisms, from mammals to prokaryotes, have an internal clock adapted to the Earth's rotational schedule of 24 h.

- By contrast, the ISS orbits within 93 min, creating a 93 min day-night cycle with different environmental stimuli.

ROS are involved in disordered sleep physiology from a variety of causes, including from noise exposure in spaceflight.

Spaceflight is Associated with Mitochondrial Dysfunction

Image Credit: NASA



Review

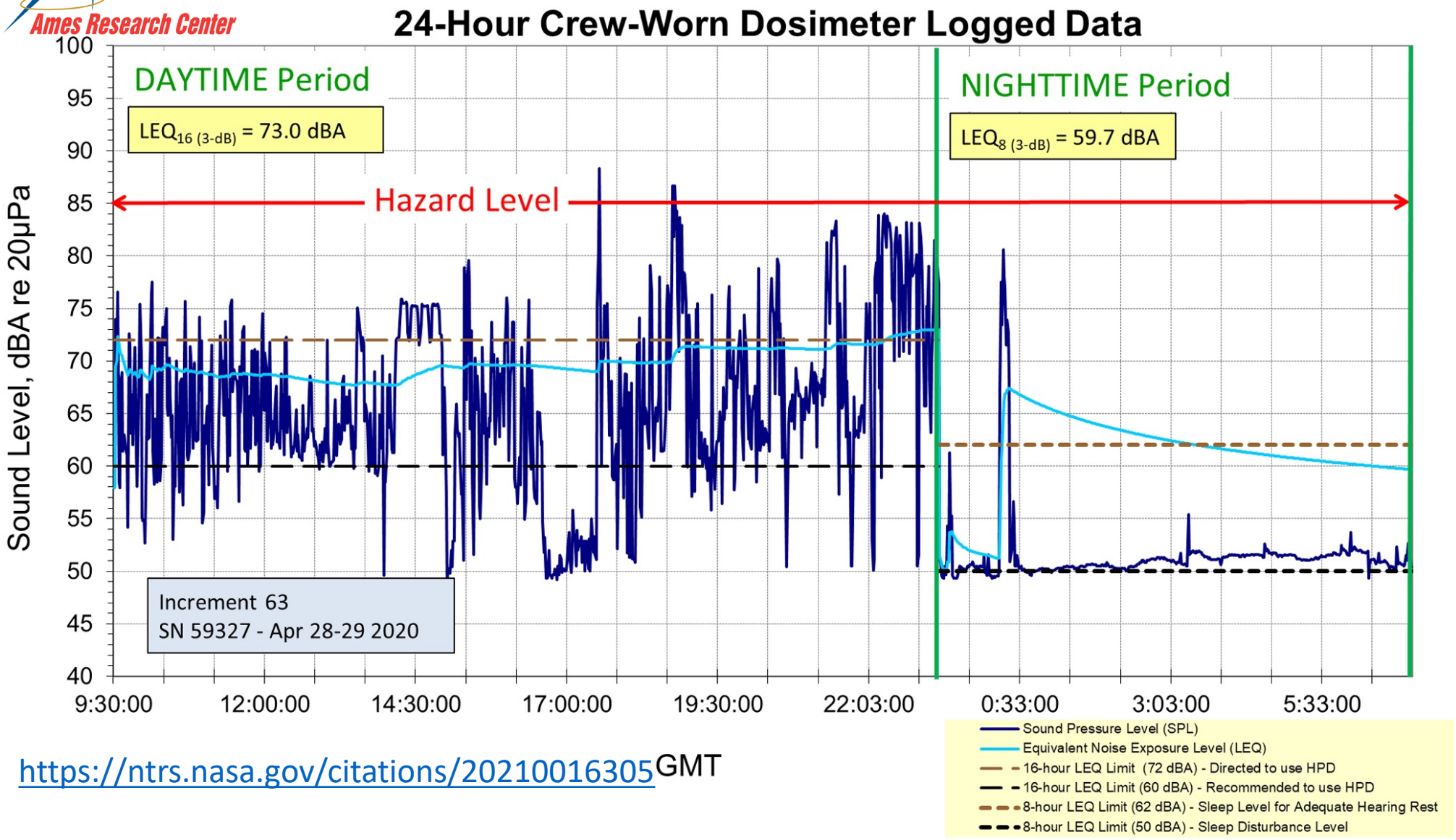
Mitochondrial stress in the spaceflight environment

Agata M. Rudolf [a b], Wendy R. Hood [a]

DOI: 10.1016/j.mito.2024.101855

Mitochondrial dysfunction is based on astronaut data, including data from the NASA Twin Study.

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https://ntrs.nasa.gov/citations/20210016305

Crew-worn Acoustic Dosimeter Logged Data.

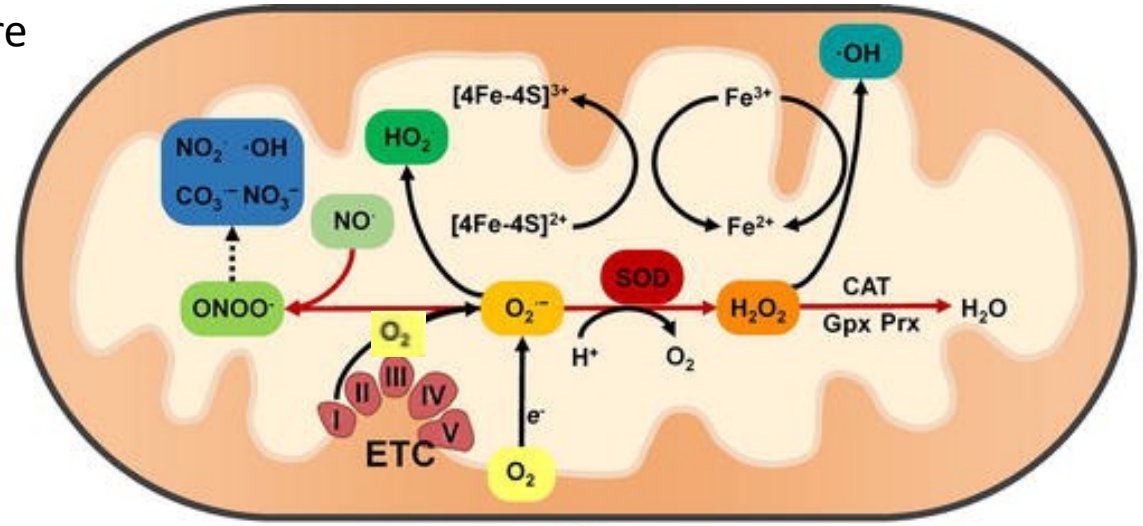
SOURCE: ISS Acoustic dosimeter data collected on April 2020, Increment 63.

Metabolic basis of timekeeping with redox switches

Reactive Oxygen Species serve a "time-keeping" function as switches of various cellular processes and physiological rhythms.

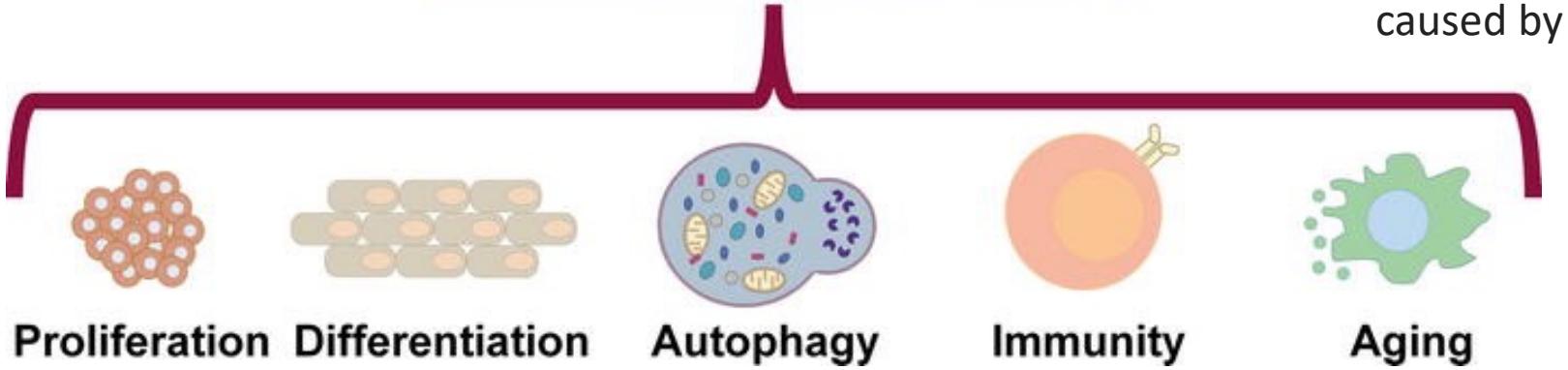
ROS (*e.g.*, hydroxyl radicals) are **produced** as byproducts of bioenergetic metabolism.

Timekeeping mechanism is based on the redox cycle, which generates **circadian rhythms conserved from bacteria to animals**.



To **counteract ROS**, cells in the CNS contain antioxidant systems, including **peroxiredoxin** and **superoxide dismutase** enzymes, to protect against **oxidative stress.**

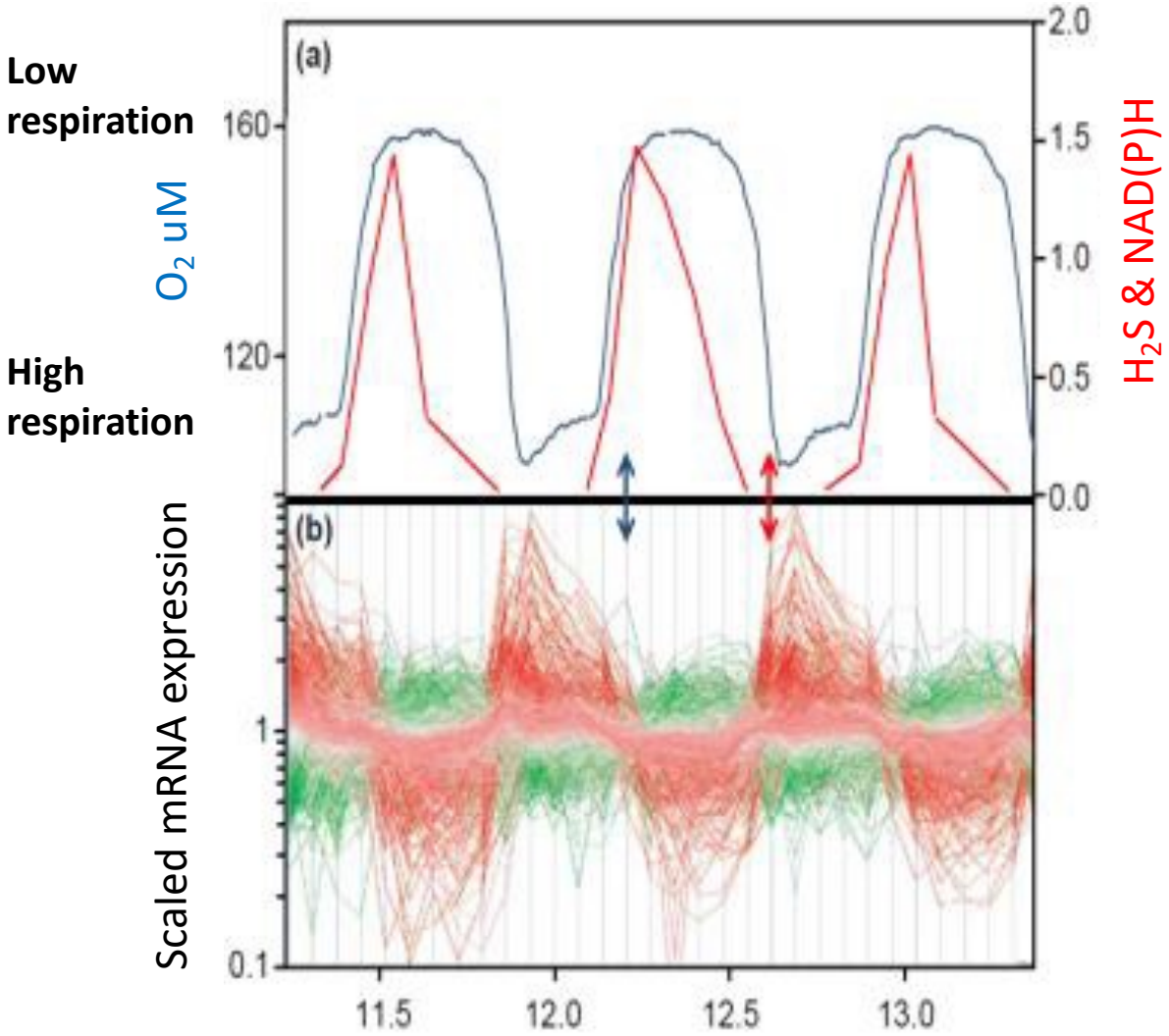
Mutants with defective peroxiredoxin enzymes exhibit increased somatic mutations caused by ROS.

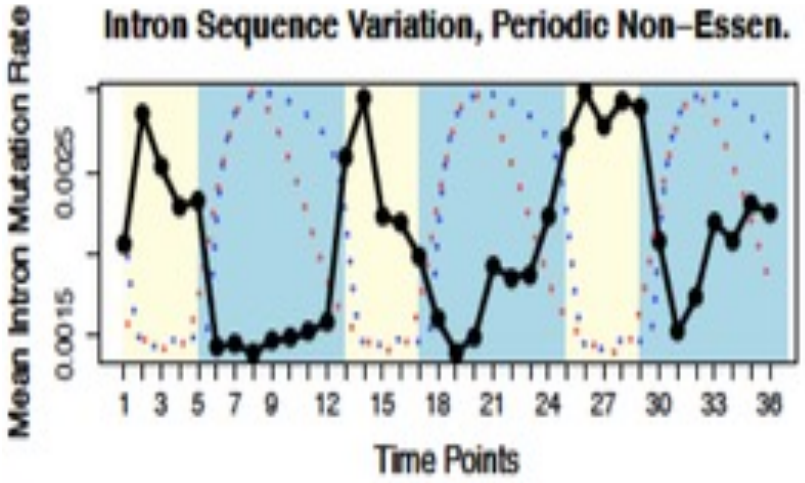


Choi, T. G. , & Kim, S. S. (2019), Free Radical Medicine and Biology.

Edgar, R., *et al.* (2012) *Nature* **485**, 459–464.

Temporal coordination of metabolic cycle is maintained by the oscillating reduction-oxidation reactions.



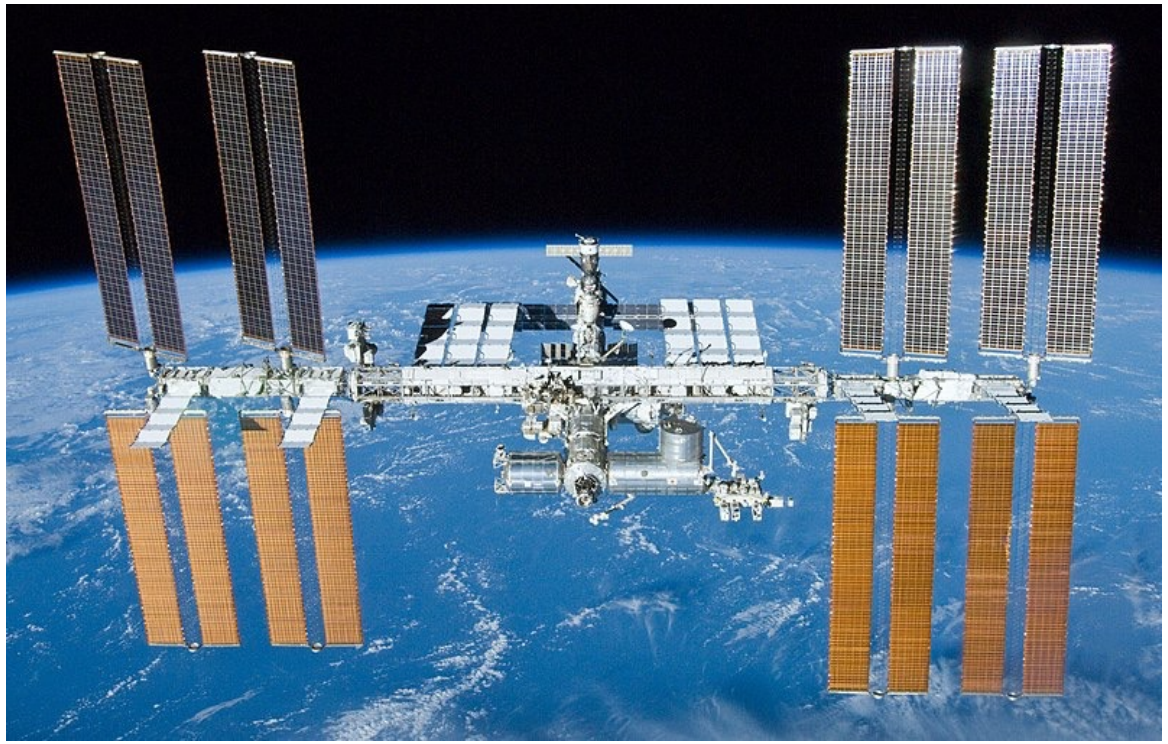


- ROS induced mutation is a fundamental genetic process that affects all species.
- ROS induced mutations may have clinical relevance for astronauts subjected to metabolic stress in spaceflight.

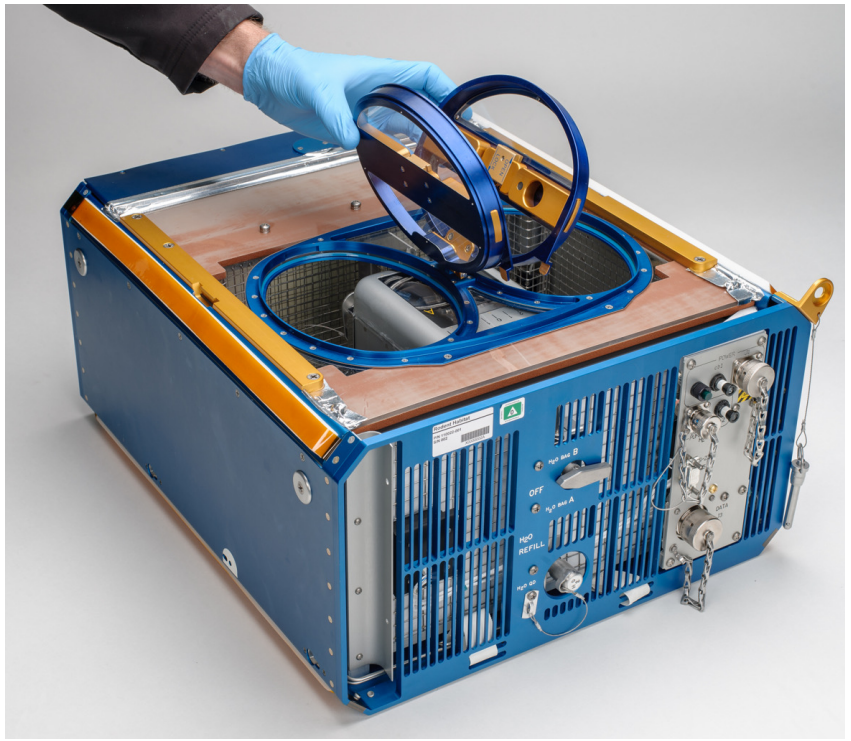
Murray D.B., *et al*. (2011) Redox regulation in respiring *S. cerevisiae*. *Biochim Biophys Acta*. 1810:945-958.

Stolc V, *et al*. (2011) PLOS ONE 6(9): e25270.

Long-duration experiments with rodents on the International Space Station



The ISS consists of pressurized habitation modules



The Rodent Research Hardware System Platform (designed at Ames)

Ames Research Center

What time is it (for sleep) in space?



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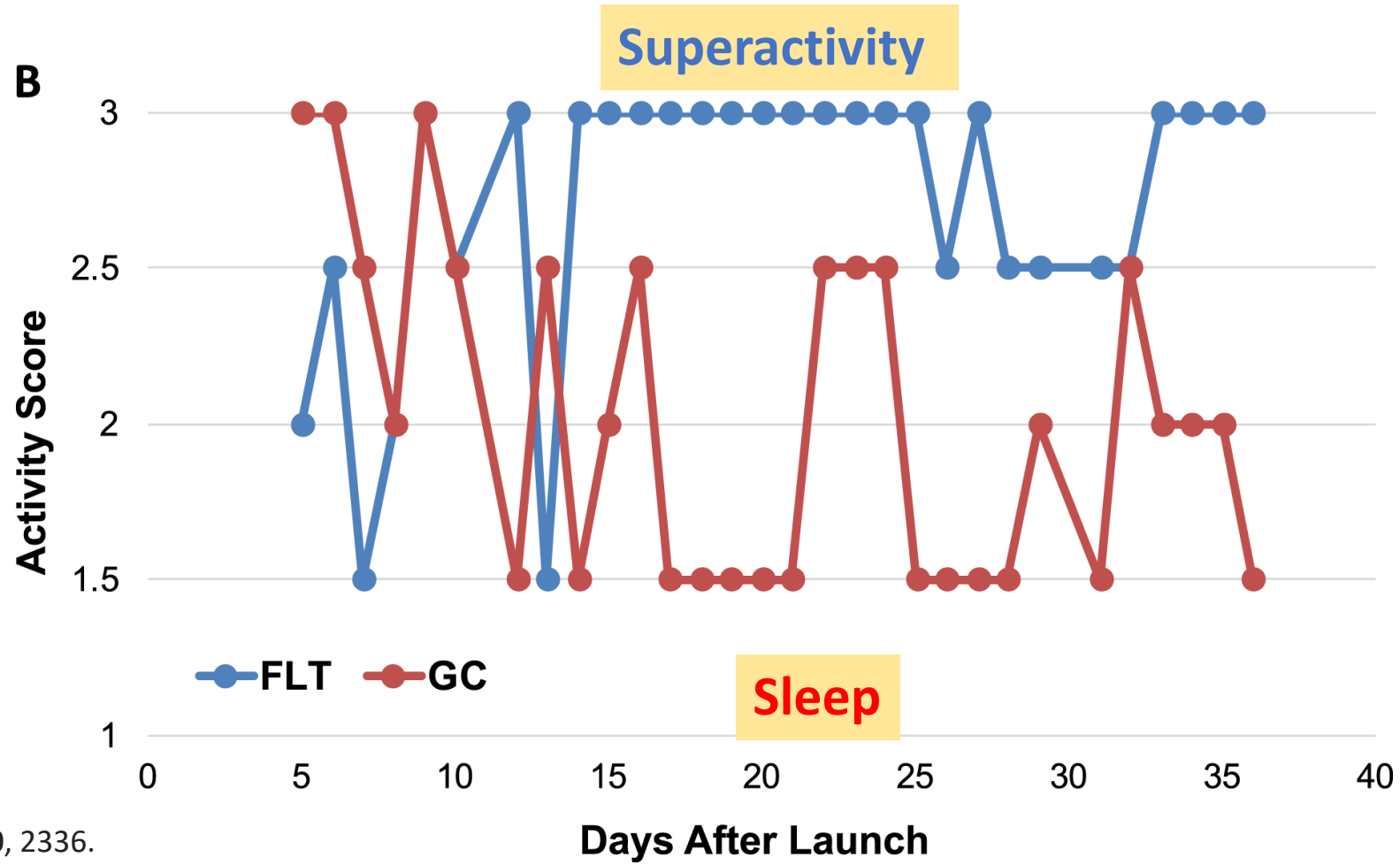
NASA

A

	Score	Activity/Behavior
Low	1	Sleeping, sitting, standing, periodic grooming
Moderate	2	Eating, drinking, slow ambulation, sustained grooming of others and self
High	3	Running laps, climbing, exploring, playing, fast ambulation, jumping

Quantitative Behavior (Activity) Assay revealed that mice became sleepless in about 14 days on the ISS.

B



Choi, S.Y., (2020) *Sci Rep* **10**, 2336.

Experiment to Evaluate Somatic Mutation Rate in Space

Ames Research Center

Method:



16 wk. old, female, C57BL/6J,
18 wk. old, female, BALB/c

Rodent Research 1, 2014

37-42d, Rodent Habitat, **ISS** → Euthasol, CD, -80° C → Thaw, Dissection → Kidney, Skeletal muscle, Eye, Liver (n = 72 each)

37-42d, Rodent Habitat, **Ground** → Euthasol, CD, -80° C → Thaw, Dissection → Kidney, Skeletal muscle, Eye, Liver (n = 72, each)

RNA sequencing library construction done at UC Davis.

Illumina HiSeq 4000

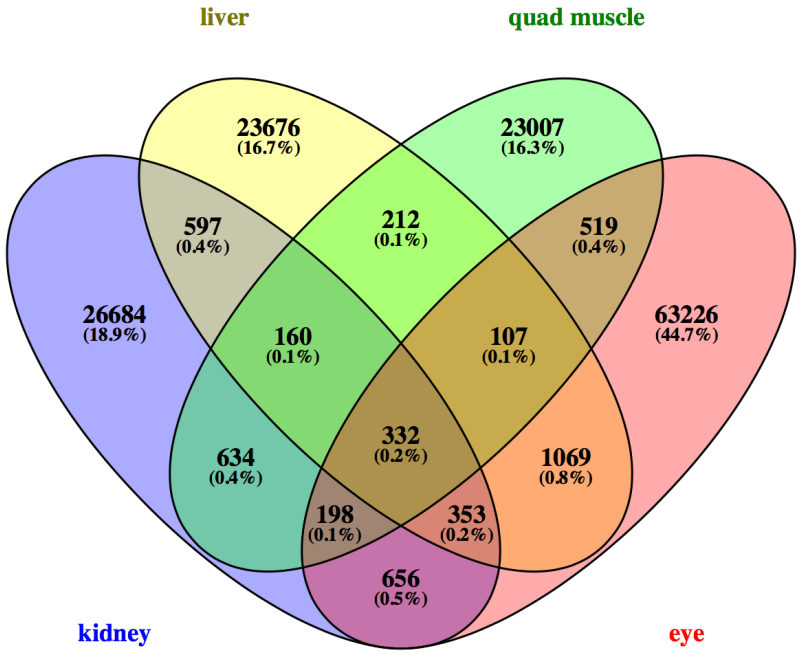
Sequence variants determined by a novel analytical pipeline developed at NASA Ames Research Center.

https://www.nasa.gov/ames/research/space-biosciences/nbisc

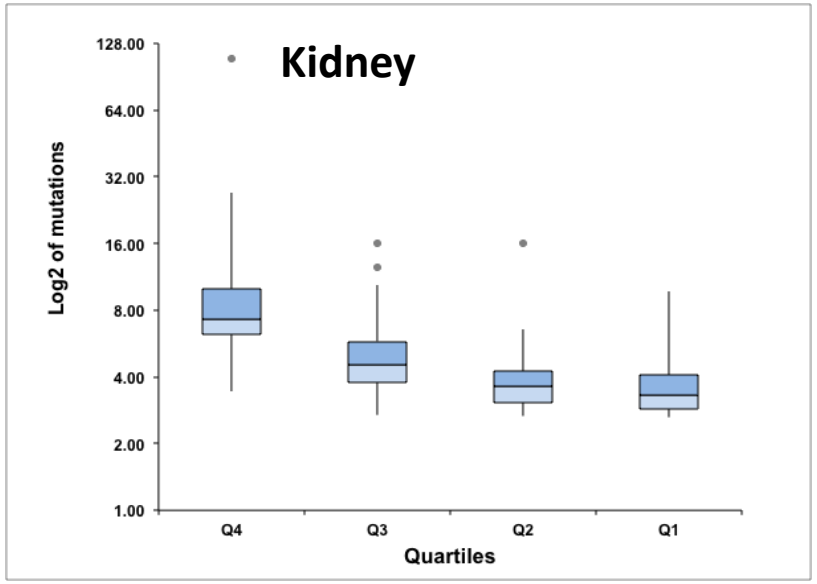
https://genelab.nasa.gov/

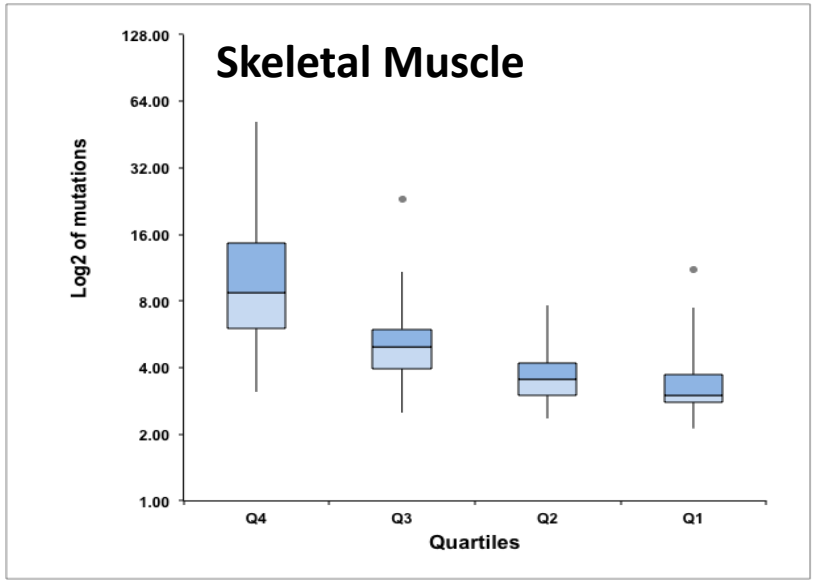
ROS-induced Hypermutation

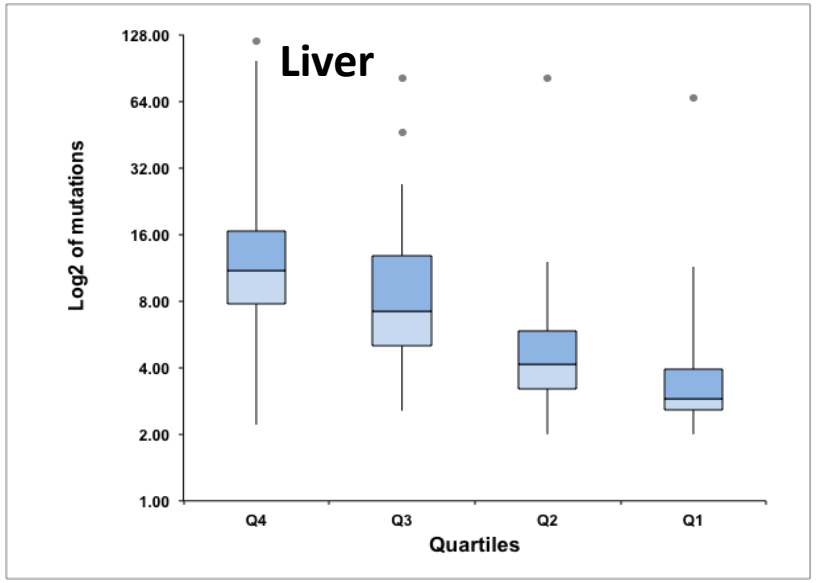
Tissue	Variants	unique SNVs	unique NSMs	unique(NSMs/SNVs)
GLDS-162 (eye)	94755	63226	22865	36%
GLDS-137 (liver)	50701	23676	6516	28%
GLDS-102 (kidney)	48577	26684	9780	37%
GLDS-103 (quad muscle)	43883	23007	7722	34%

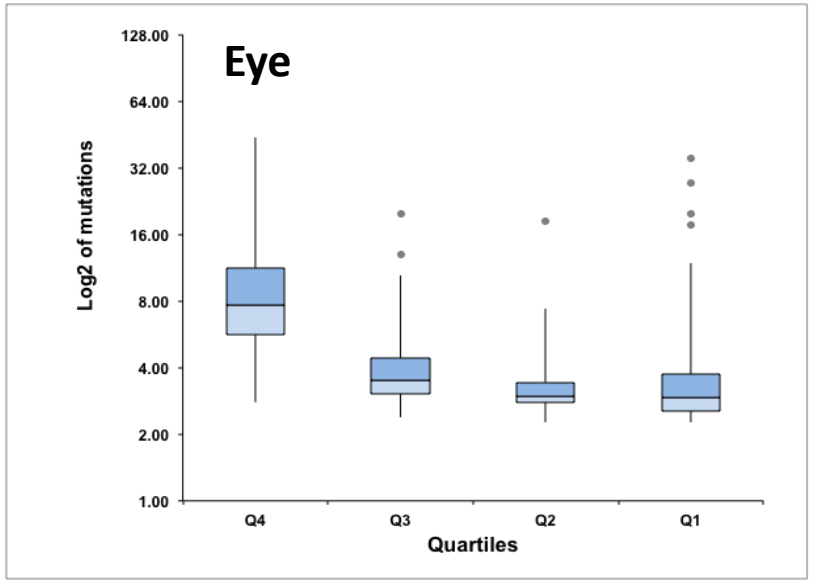


Mutation increased by induced RNA transcription during spaceflight on the ISS relative to ground controls







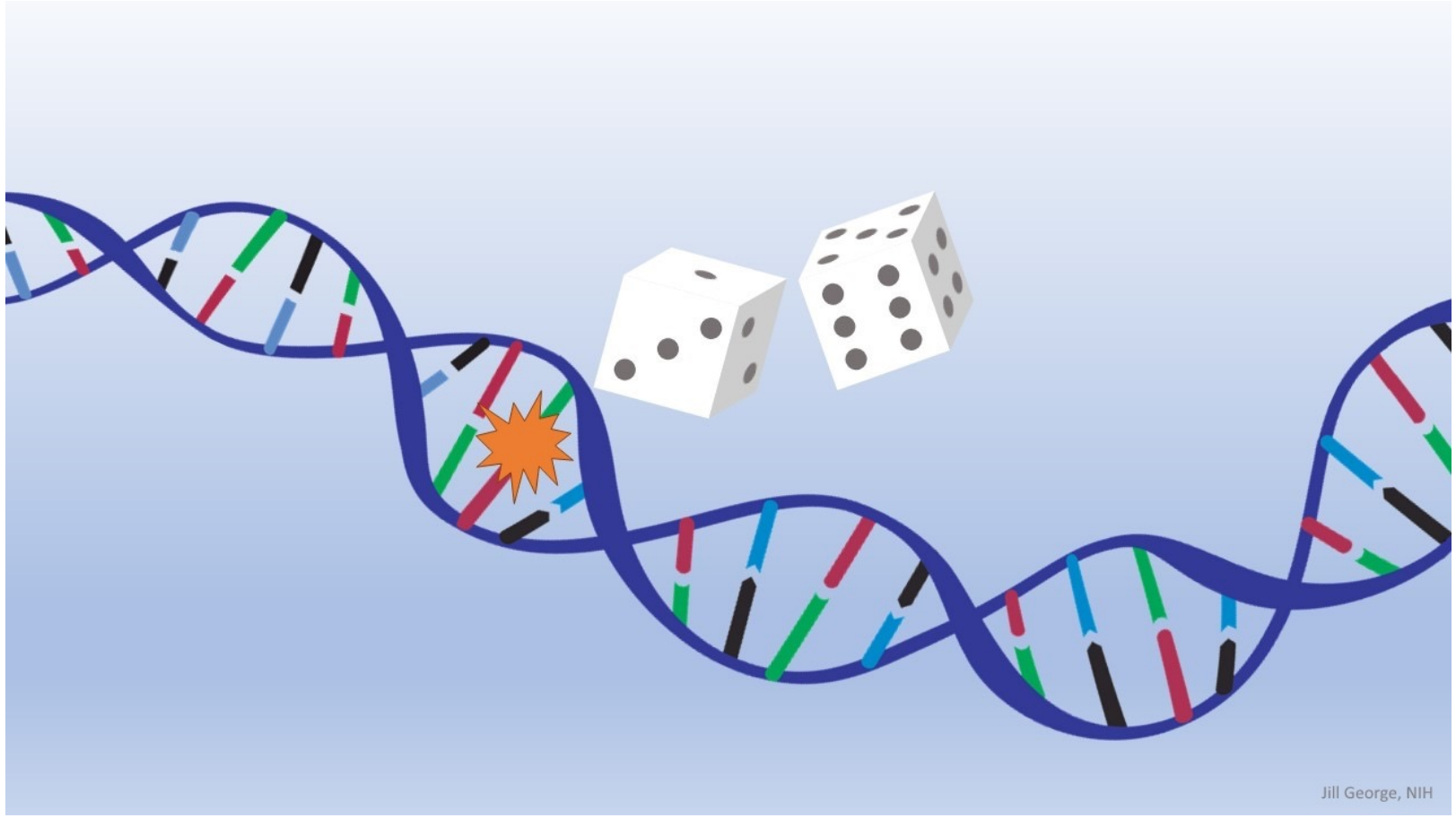


- Average mutation frequency 6.69 x 10^{-6} per base pair (bp) for all differentially expressed genes (in total 18,140 genes) **on the ISS**,

vs

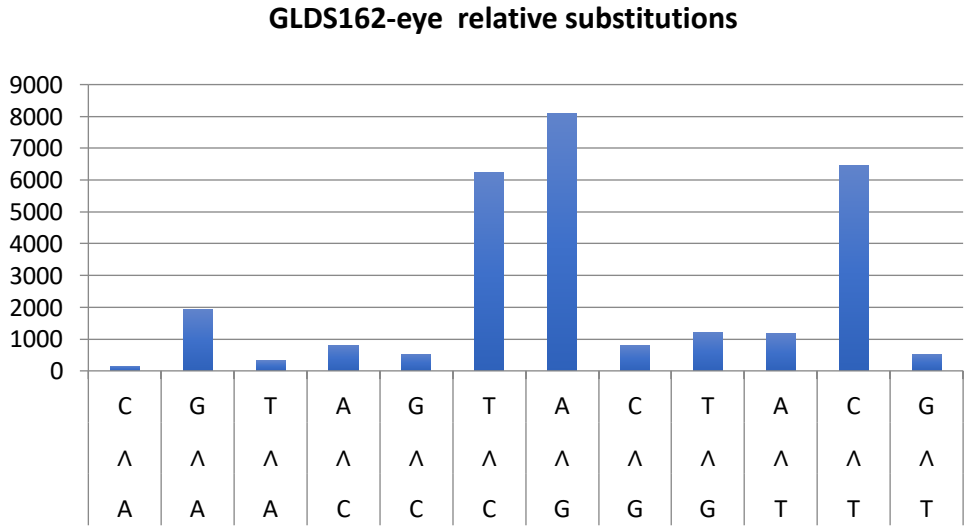
- Average mutation frequency 3.79 x 10^{-7} mutations per bp (1.7 mutations per genome per generation) **on Earth**.

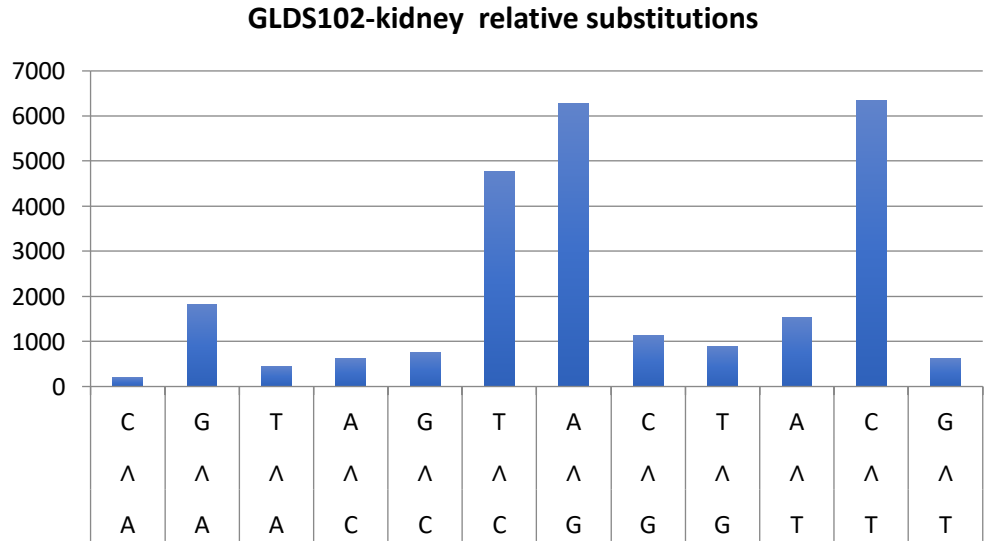
Are ROS-induced Somatic Mutations Completely Random?

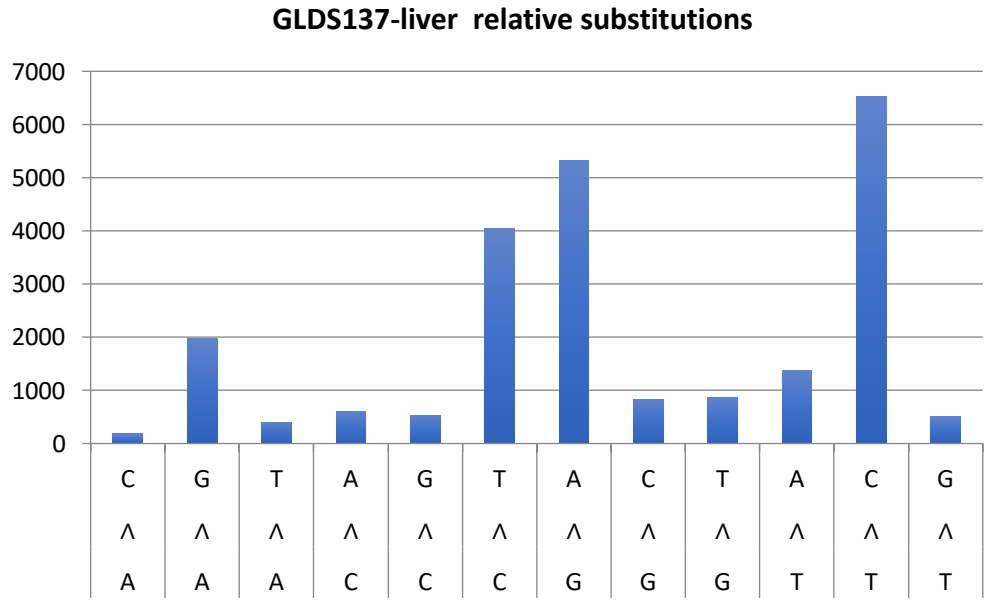


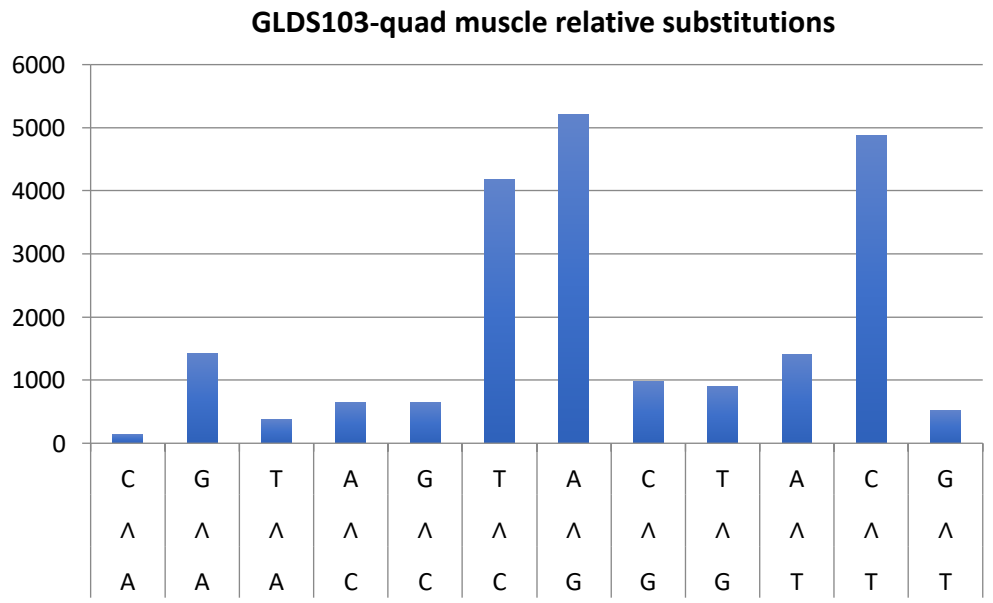
Are As, Ts, Cs and Gs being altered with the same frequency?

ROS-induced base substitutions, for each of the four tissue types









Guanine is the most easily oxidized of the nucleotide bases, a process that can occur because of endogenous production of reactive oxygen species (ROS).

Summary

- Reactive oxygen species play a critical role in the CNS in both normal development and adult physiology and play a critical role in pathophysiology as well.
- Mitochondria play a crucial role in regulating the body's internal clock through ROS, impacting sleep patterns. Balanced ROS levels are essential for maintaining proper sleep-wake cycles. Excess ROS in the central nervous system can disrupt metabolic functions, contributing to sleep disorders and dysregulation of circadian rhythms.
- The range of pathologies that NASA is concerned about for astronauts bears a striking resemblance to the range of pathologies caused by metabolic dysfunction, indicating that ROS is the likely common link.
- This heightened oxidative stress from metabolic dysfunction may contribute to mutations and cellular damage, especially in the CNS, *via* mechanisms that link spaceflight pathophysiology with terrestrial pathophysiology.