Molecular Changes Induced by Microgravity

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Introduction

Area of Research

NASA Human Exploration and Operations (HEO) Mission Directorate for Space Life and Physical Sciences Research & Applications

This research investigates

The characterization of TEs for facilitating horizontal gene transfer and DNA repair in rotifers.

Extreme Environments - Space

Astronauts on ISS - Physiological & Molecular Changes - Adaptation

- Scientific research experiments - Astronauts
- Model Organisms: Vertebrates & Invertebrates

Why?

Space Travel & Medical Advances & Treatments on Earth

JAXA - Rice Fish (Medaka) in Space

YEAR	MISSION	AGE AT LAUNCH	DAYS ON ISS
1994	International Microgravity Laboratory mission	Adult	15 Days
2012	Medaka Osteoclast research	6-weeks old	2 Months

RNA-seq analysis of tissues – response to the microgravity environment vs. Control

Background

- **Medaka Fish**
- JAXA & Tokyo Institute of Technology
- ISS & Ground
- RNA sequencing data
- DNASeq

↑ space flight ↓ bone density

Molecular Mechanism?
Osteoclast Activation

→Background for Bone Development

Osteoblasts
- Make Bone
- * Propogating as a result of
- * Growth Factors & Bone Mechanical Stress

What are Osteoclasts?

Counterbalance to osteoblasts = **osteoclasts** = the bone reabsorbing cells.

Osteoclasts
- Make & Secrete Digestive Enzymes
- * Breaks up/Dissolves Bone Tissue
- * Growth Factors & Bone Mechanical Stress

Medaka Significance→ Bone Development→ looking at vertebra formation & identified **presence of osteoclasts**

Discovery→Hypergravity affects osteoclast re-absorbing activity→examine osteoclasts in microgravity→ ISS→Testing→Histological & Gene Expression Analysis

Objective

Basis for our research→ JAXA gene datasets

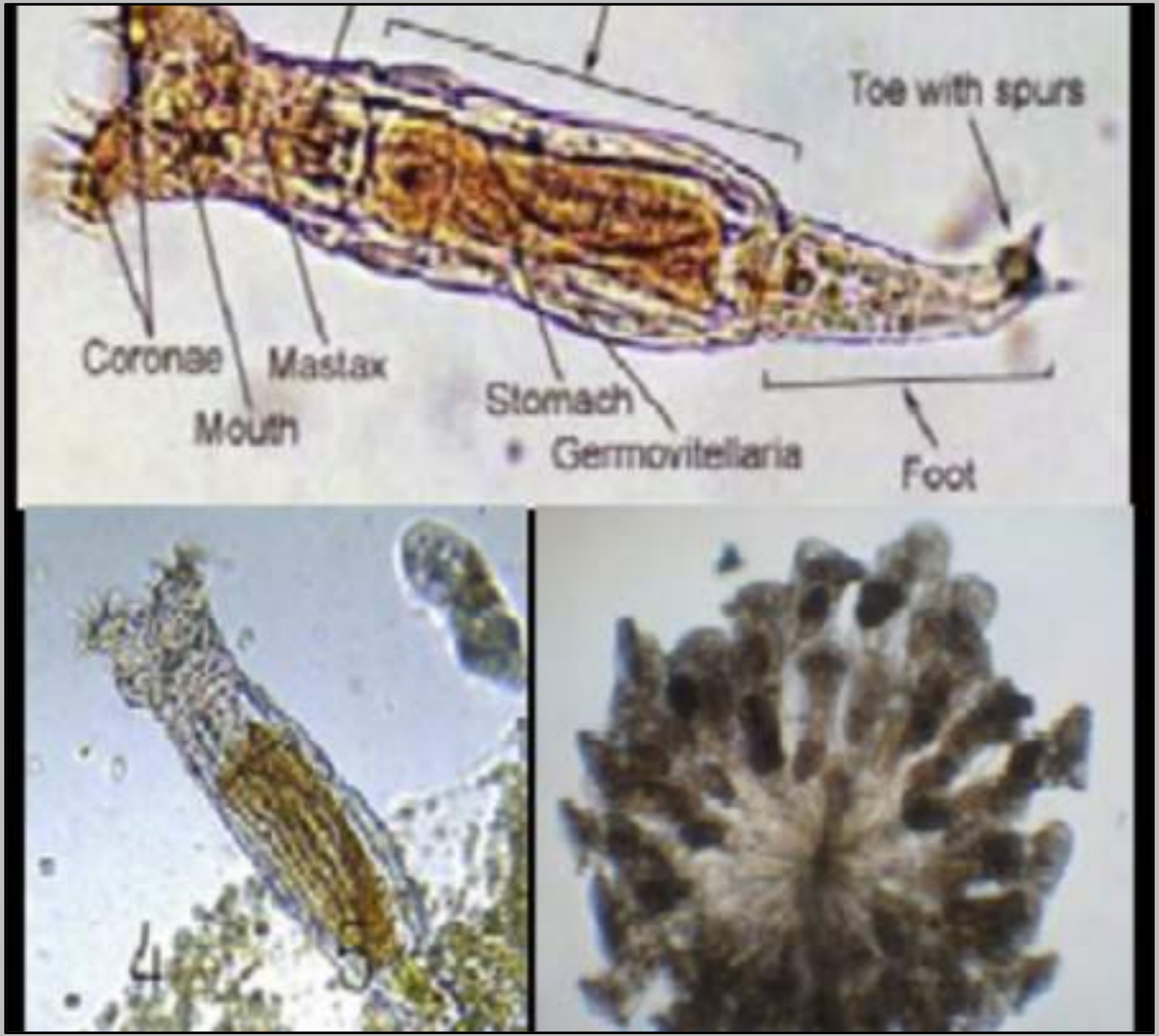
Focus:

- Transposons
- Molecular mechanisms that multicellular organisms in response to environmental stress to increase survival→
- Induce horizontal gene transfer following microgravity exposure→
- The effect on cell structure and growth
- Relevance to human space exploration

Project Identifies

- Potential molecular mechanisms of horizontal gene transfer
- The regulators of TEs, involved in disease risk:
- such as cancer
- applications on Earth
- long-term space travel

Model Organism



Bdelloid rotifers

- Latin for “wheel bearer” due to crown of cilia around the mouth of the rotifer
- Complex micro-invertebrates
- Recognizable tissues and organs
- Length ~ 0.5 to 1.0 mm
- A colony occupies ~1 mm^2 of surface area
- 40-50 individual organisms/colony

Where do you find Rotifers?

- Ponds, fresh water, some saltwater species, moist soil & thin film of water surrounding the soil

- **The Process**
- Water dries up
- Rotifers dehydrate (shrink)
- Surviving for decades without water
- Ability to travel via wind to new pools of water

Rotifers can successfully survive extreme environments.

http://theconversation.com/these-sex-starved-creatures-scavenge-new-genes-from-other-pondlife-56913

Epigenetics

- Dutch scientist Antonie van Leeuwenhoek (rotifers using microscope)
- procreate asexually
- No one has ever spotted a male
- Females lay eggs→ but no meiosis
- All eggs contain genomes identical to female
- 2 copies of each gene (diploid), but are different
- Homologous chromosomes→have been differently rearranged→can't pair at meiosis.

Conclusion: Rotifers originated as a hybrid between two species whose genes and genomes diverged 60 million years ago

Survivability

- Capable of surviving extreme environmental stress

- Engulfing foreign DNA through horizontal gene transfer

Process **Dehydration to rehydration**

- In just a few hours

- **DNA sequencing shows:**
- They are incorporating genes from other rotifers of the same or different species, or even from fungi and bacteria.

Positives - Efficiently repair their DNA, broken in many places during desiccation

Why Use Rotifers?

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Why Use Rotifers?

- Detect role of TEs as a molecular mechanisms underlying horizontal gene transfer.

- Genes acquired by the rotifer.

- Potential novel biotechnology

Why Use Rotifers?

- **New DNA for rotifers**
- DNA Sequencing
- ~ 8% of the genes looked foreign
- Some genes were typical of fungi or bacteria, and endowed the rotifer with handy new properties such as breaking down toxins or using newly engulfed food .
- This "horizontal transfer" between rotifers and other organisms is ancient & ongoing.

HORIZONTAL GENE TRANSFER
(occurs as a result of)

Mobile Genetic Elements

- Extra Chromosomal Genetic Material: Plasmids
- Bacteriophages*: Bacteria Infecting Viruses
- Jumping Genes*: Transposons

Genetic Elements Transfer

Prokaryotes & Eukaryotes

In Prokaryotes

Transformation

Take up fragments of DNA (can be in the form of plasmid)

Conjugation

Genetic material exchanged in a temporary union between 2 cells (can be the transfer of a plasmid or a transposon)

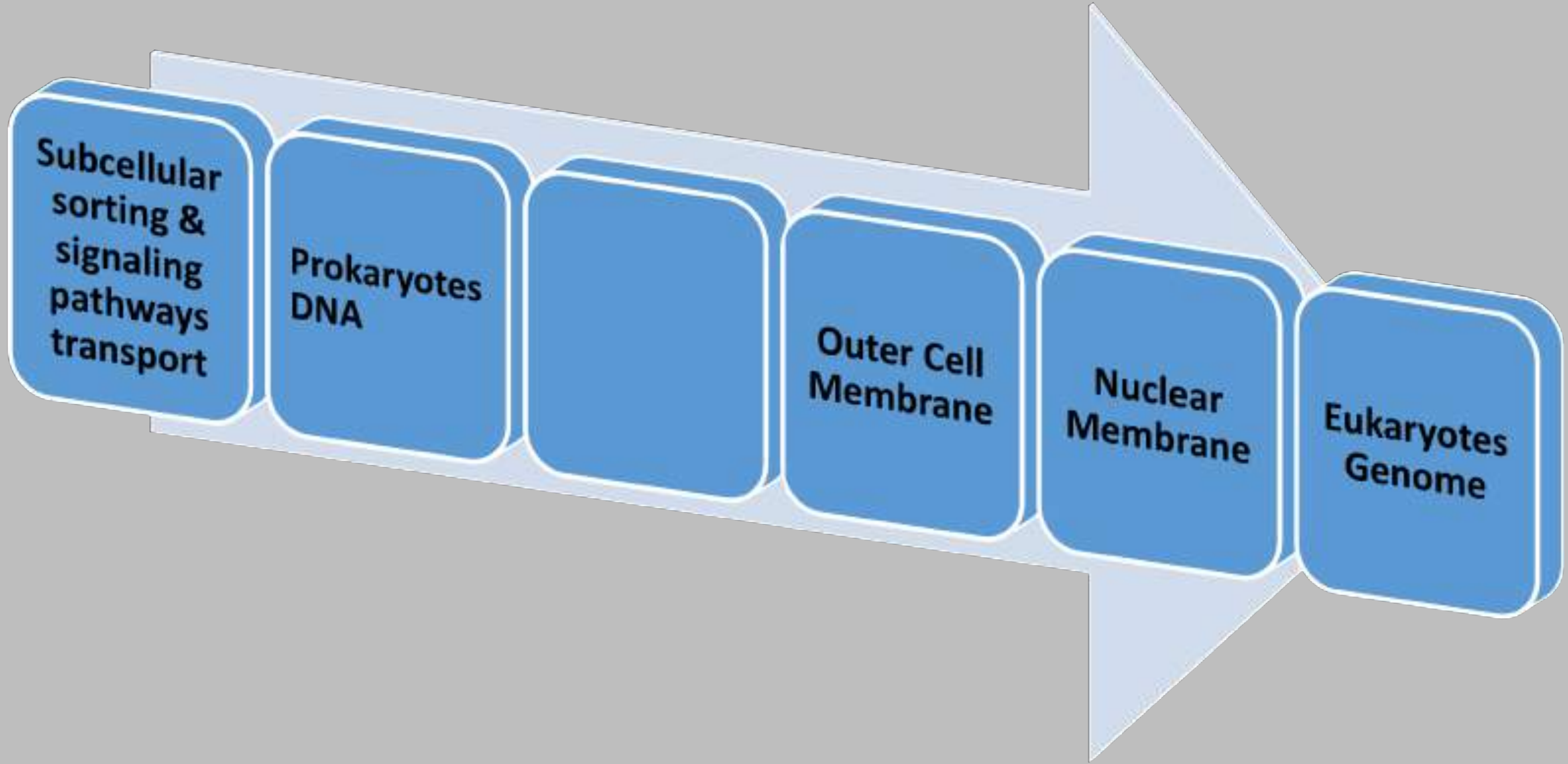
Transduction

DNA transferred from 1 cell to another by bacteriophage.

Horizontal Gene Transfer→ newly acquired DNA→ incorporated into the genome of the recipient→by **recombination** (regrouping of genes – native or new & are **homologous**) or **insertion** (**new & no homology w/existing DNA**)

HORIZONTAL GENE TRANSFER

Eukaryotes



New DNA for Rotifers

Purpose

- Foreign DNA is spread all over the rotifer genome
- How? Dehydration may makes holes in cell membranes. Rehydration & horizontal gene transfer
- Efficient mechanism
- Repairing double stranded DNA breaks
- Dehydration ideal for incorporating foreign DNA into genome

Incorporation not just anywhere in the genome.
Lines up with the appropriate DNA sequence & recombines.

Unique to Rotifers?

- Ability to take up & use DNA from environment
- Not unique to rotifers→**Bacteria**
- Bacteria reproduce by fission to make clones of genetically identical cells.
- Bacteria can take up DNA from another bacterial strain,
- Swap it for the resident gene & express a variant protein in the wild.
- This "DNA transformation" provided the first evidence that genes are made from DNA.

Rotifers: Adapt & Acquire.→ genetic variation is essential for evolutionary success

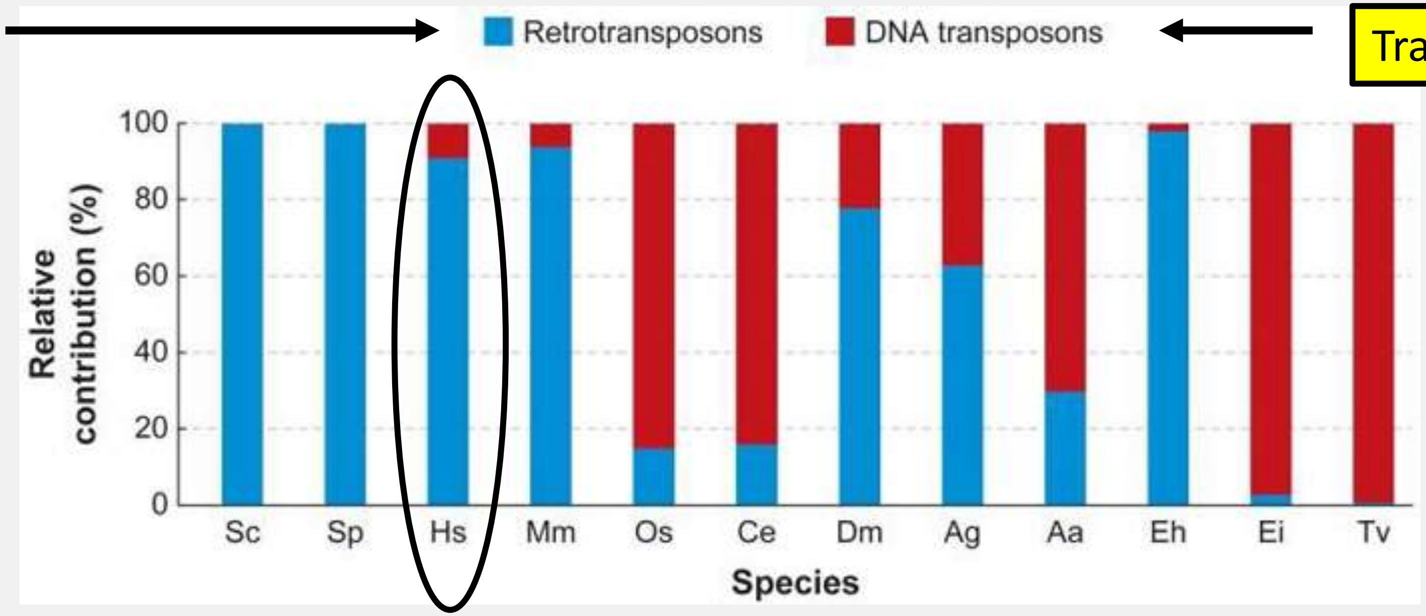
What are Transposable Elements (Transposons)?

- Genetic elements that reside in all prokaryotic and eukaryotic genomes
- Bacteria to Rotifers to Humans
- Natural “genetic engineers” incorporate new DNA into the genome
- ~ one-third of the human genome (extensive part of "our DNA is not entirely our own“)

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2014). *Essential Cell Biology.* New York, NY: Garland Science

Retrotransposons – Repetitive DNA Fragments that are Reverse Transcribed from RNA

Transposons – Genomic DNA



Both move by a "copy and paste" mechanism

Retrotransposons – Reverse transcribed from RNA

Transposons – from DNA

In Contrast - RNA copies are **transcribed back into DNA** — using a **reverse transcriptase, (an enzyme)** (found abundantly in genomes of plants & animals) & are inserted into new locations in the genome.

Reverse trancriptase example:

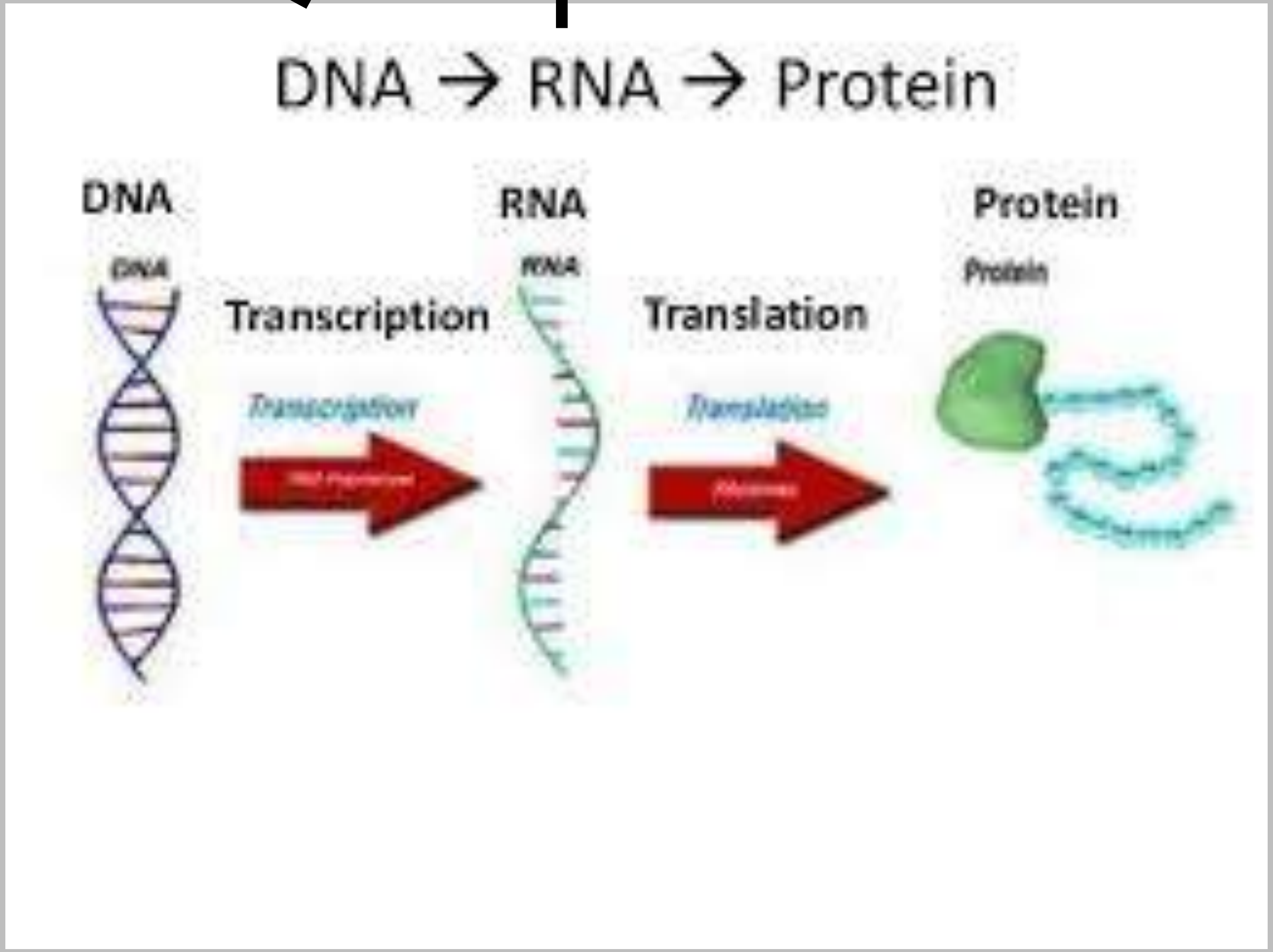
Telomerase, an enzyme that adds nucleotides to telomeres (such as cancer cells) found in many eukaryotes (including humans) & carries it's own RNA Template for DNA replication.

Figure 1: The relative amount of retrotransposons and DNA transposons in diverse eukaryotic genomes

This graph shows the contribution of DNA transposons and retrotransposons in percentage relative to the total number of transposable elements in each species. (Sc: Saccharomyces cerevisiae; Sp: Schizosaccharomyces pombe; Hs: Homo sapiens; Mm: Mus musculus; Os: Oryza sativa; Ce: Caenorhabditis elegans; Dm: Drosophila melanogaster; Ag: Anopheles gambiae, malaria mosquito; Aa: Aedes aegypti, yellow fever mosquito; Eh: Entamoeba histolytica; Ei: Entamoeba invadens; Tv: Trichomonas vaginalis.)

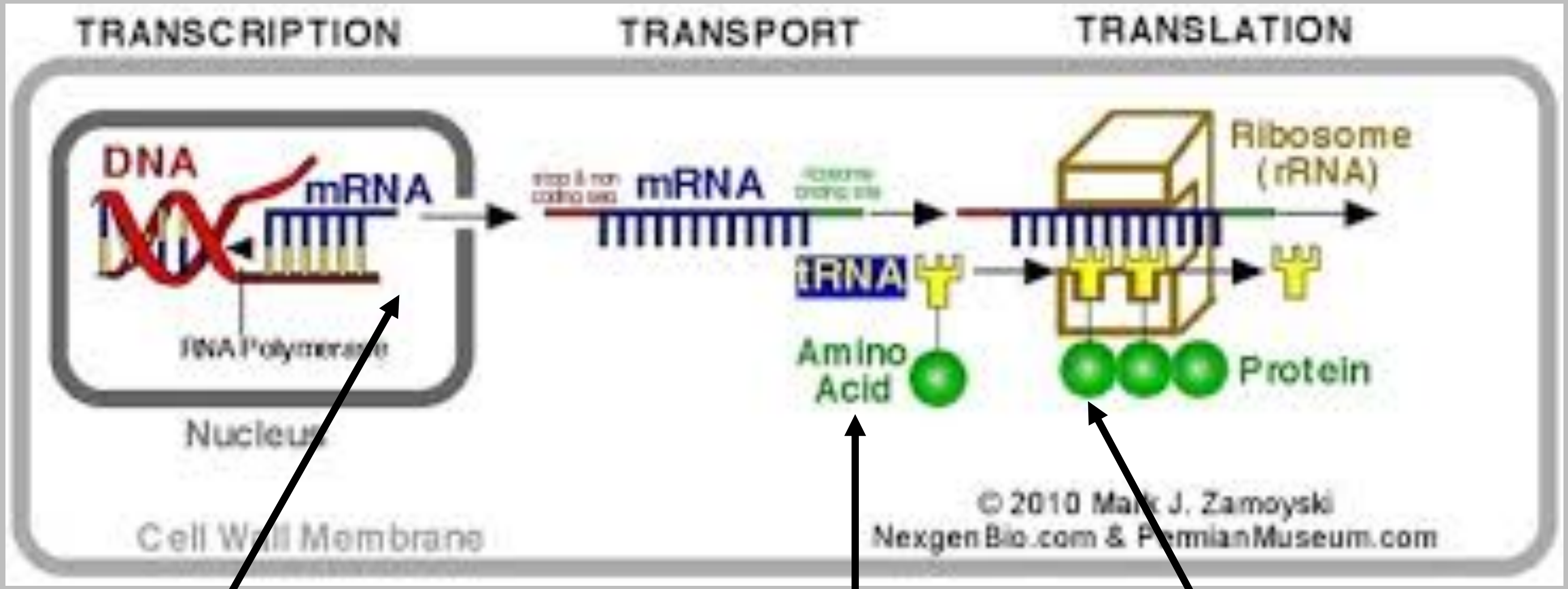
Transposons – Genomic DNA

Retrotransposons –Repetitive DNA Fragments that are Reverse Transcribed from RNA



https://www.slideshare.net/RosioDeLeon/dna-and-rna-55344251

mRNA combines with **rRNA** & **tRNA**
translates the **mRNA** code into **amino acid** sequence = a **protein**



mRNA made from DNA
- Carries genetic info from the Nucleus

tRNA brings Amino Acid to Ribosome

Incorporates into growing protein

What are Transposable Elements (Transposons)?

- Unknown how environment stress triggers horizontal gene transfer→environmental stress activates transposons
- In a genome TEs can modify its position
- May either correct or cause mutations
- Jumping Genes, 1st identified by geneticist Barbara McClintock

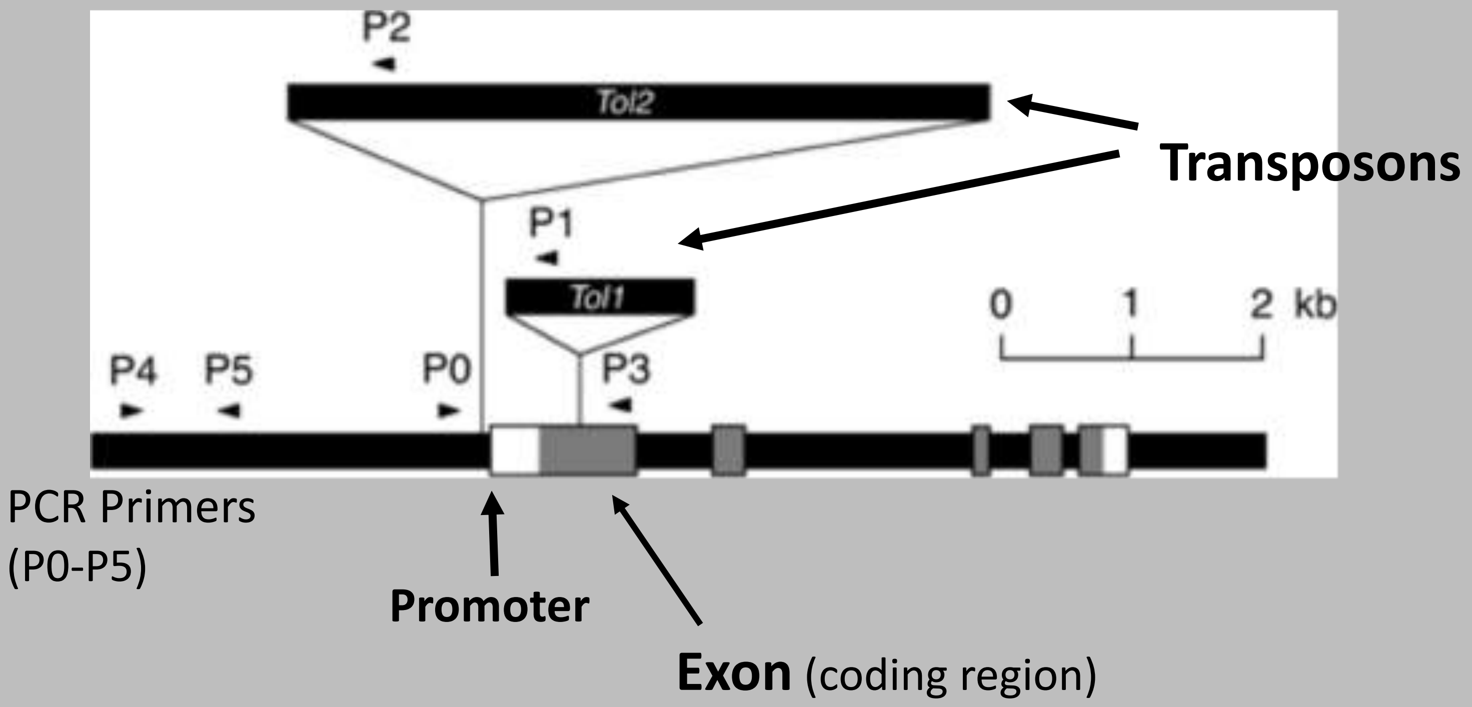
What Do Jumping Genes Do?

- ~half of the human genome is made up of TE's
- Significant portion of these genes are retrotransposons
- Depending on where it lands, can cause a **<u>mutation</u>**
- → hemophilia
- →cancer

Transposons – Not Always Destructive

Transposons can drive the evolution of genomes→ facilitating the translocation of genomic sequences→shuffling of exons & repairing double-stranded breaks

Medaka Fish – Tyrosinase Gene



Transposon Tol2 – linked directly to pigmentation in the Medaka Fish

From: Vertebrate DNA Transposon as a Natural Mutator: The Medaka Fish Tol2 Element Contributes to Genetic Variation without Recognizable Traces

Mol Biol Evol. 2006;23(7):1414-1419. doi:10.1093/molbev/msl003

Medaka Fish - Oryzias latipes

- Where *Tol2* transposon hopped out "cleanly" (i.e., without removing other parts of the genomic sequence), the fish were albino.
- Where *Tol2* did not cleanly hop from the regulatory region, the result was a wide range of heritable pigmentation patterns (Koga *et al*., 2006).

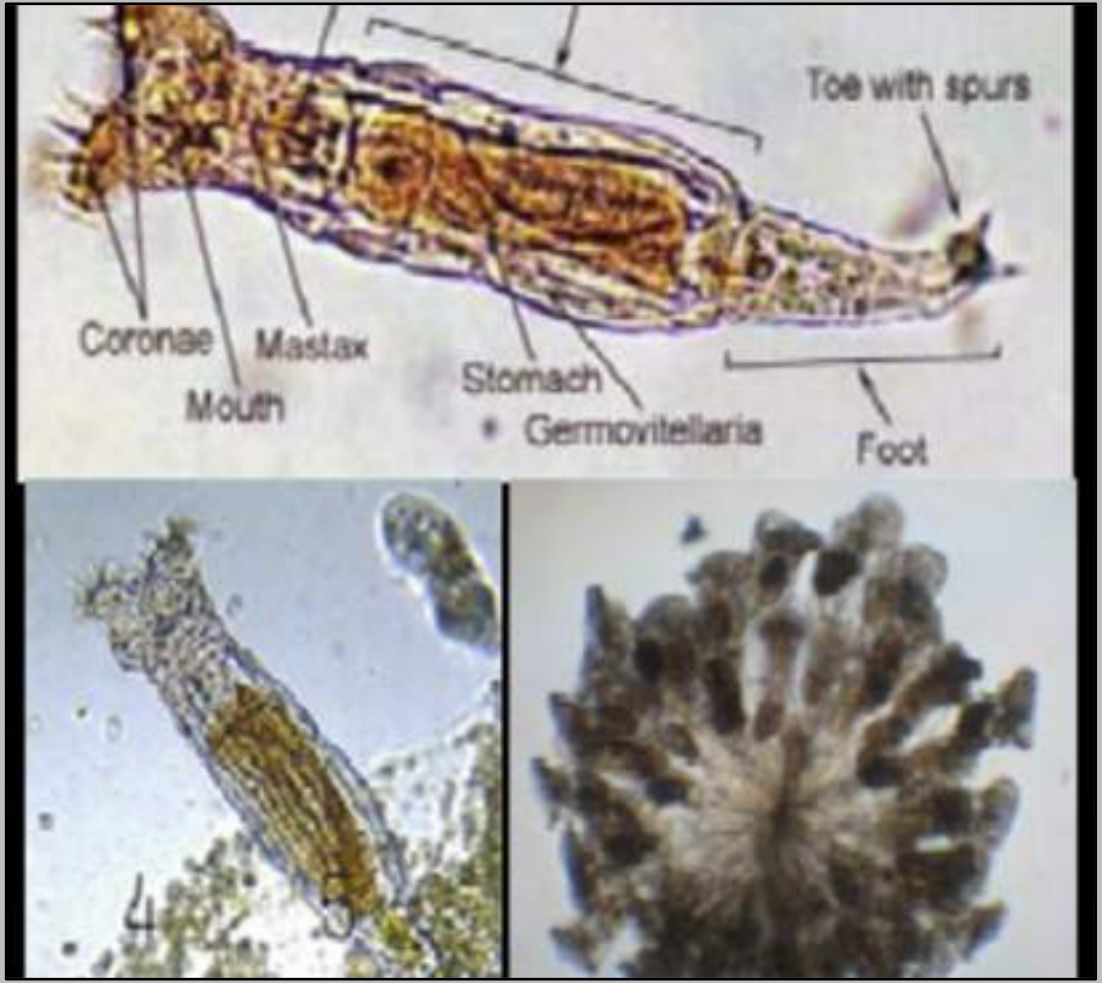
Koga, A., *et al*. Vertebrate DNA transposon as a natural mutator: The medaka fish *Tol2* element contributes to genetic variation without recognizable traces. *Molecular Biology and Evolution* **23**, 1414–1419 (2006) doi:10.1093/molbev/msl003

Medaka Fish Testing

- DNASeq – horizontal gene transfer - Exchange of genetic materials
- RNASeq – Gene transcripts

https://flickr.com/photos/120774285@N06/19079702612/

Rotifers - Background



- Microscopic multicellular eukaryotic organisms
- Adaptive response to extreme environments (such as dehydration and ionizing radiation)
- Asexual reproducing fresh water invertebrates

Rotifers Research Hypothesis

Microgravity will expedite the role of environmental stress in understanding horizontal gene transfer.

The Research Will Examine & Address

The capacity of TEs in genomic adaptations
Potential treatment of human disease

Rotifers – Extremes & Space Research

At any life cycle stage bdelloid rotifers

- Survive environmental stresses by entering anhydrobiosis
- Rapidly desiccate in a wide range of temperatures (-273 ºC to +151 ºC)
- Retain viability for nearly ten years

As rotifers recover from anhydrobiosis→May adopt foreign DNA from other nearby organisms (bacteria, fungi, and plants) horizontal gene transfer

Objective

Test the hypothesis: bdelloid rotifers in simulated microgravity will facilitate horizontal gene transfer by altering expression of transposons and transcripts.

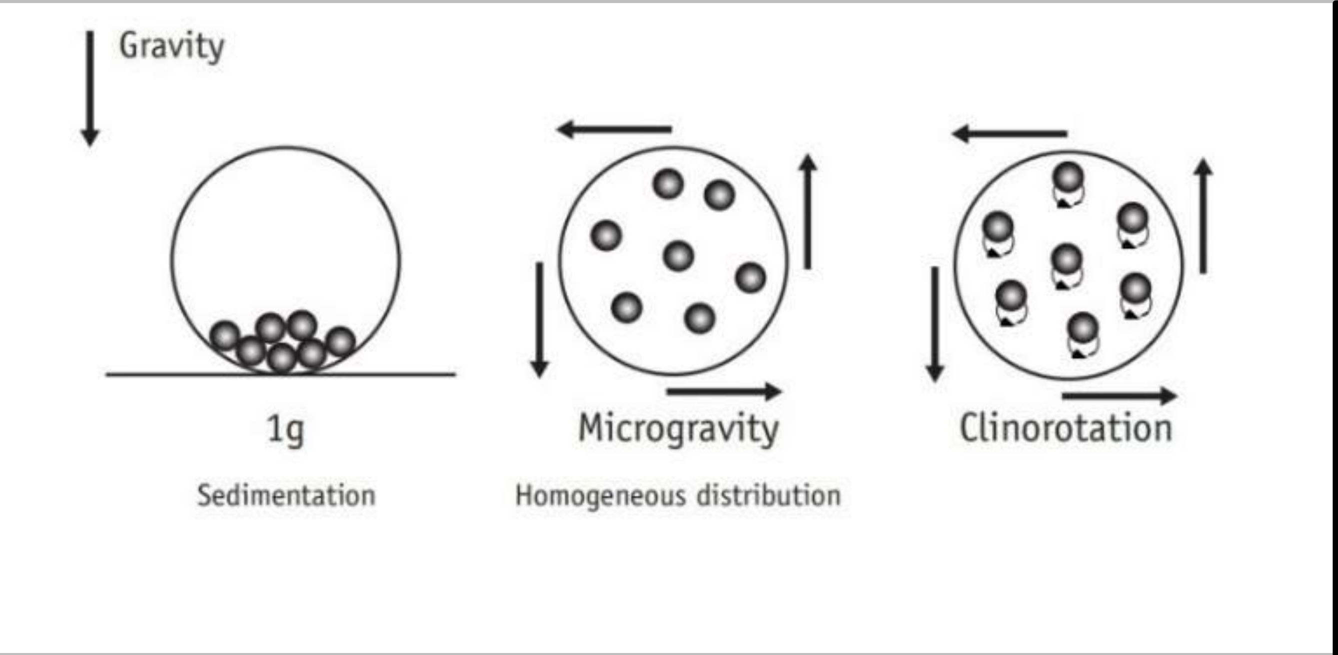
Biological Innovative Concept

Epigenetics: Environmental-induced transposons expression as a molecular mechanism→ driving horizontal gene transfer

As rotifers recover from anhydrobiosis→May adopt foreign DNA from other nearby organisms (bacteria, fungi, and plants) horizontal gene transfer

Methods

Control vs. Simulated Micrograviity (clinostat & centrifuge)



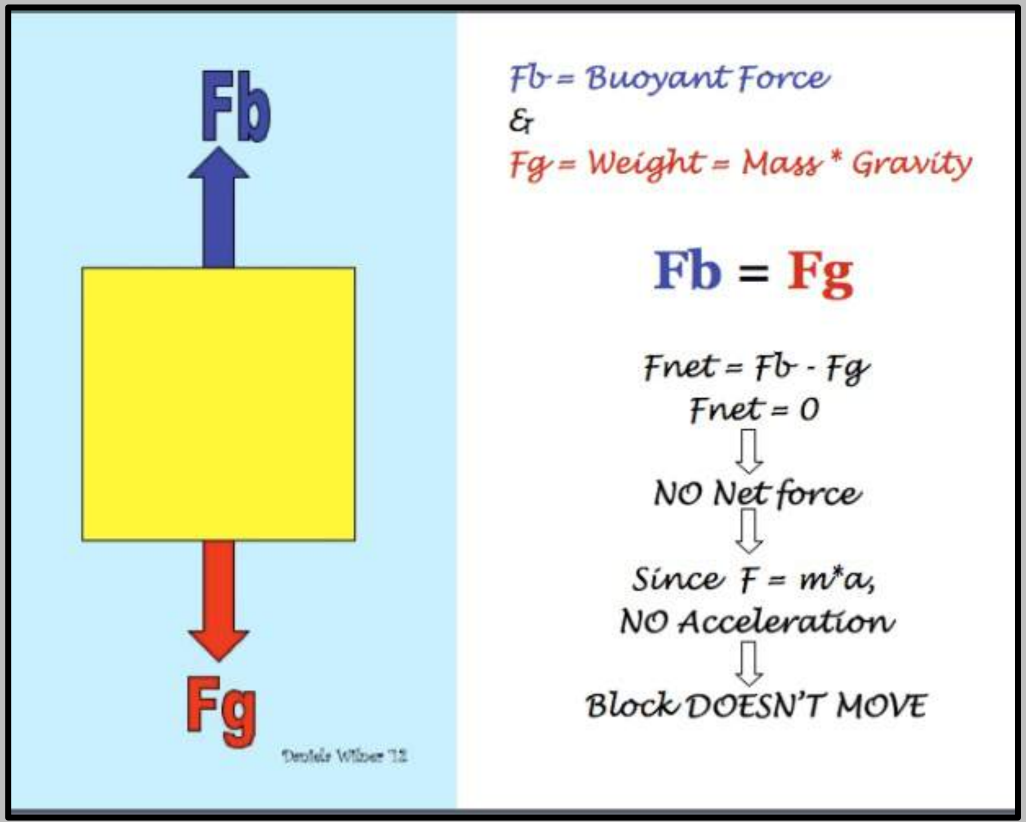
Negatives: Rotation - remove force due to gravity? No

Positives: the constant rotation results in the g-vector that is time-averaged approx. to near zero-g.

Simulated Microgravity

Freefall – drop tower, parabolic flight

Neutral Buoyancy



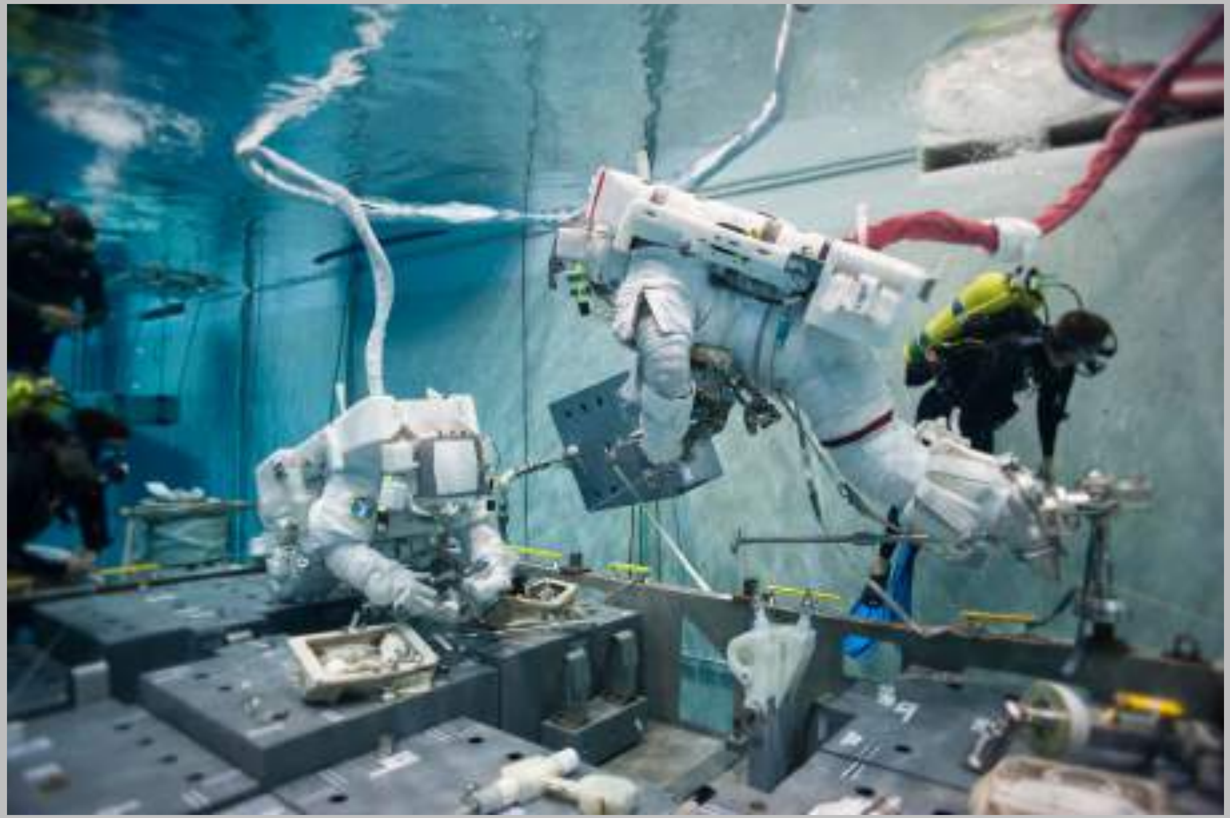


Photo credits: https://upload.wikimedia.org/wikipedia/commons/5/5d/Terry_Virts_simulates_extravehicular_activity_in_the_Neutral_Buoyancy_Laboratory.jpg and https://i.stack.imgur.com/fdqr9.jpg

https://flickr.com/photos/69778419@N02/6915313113/

Neutral Buoyancy

Methods (continued)

Transposon Enrichment Set Analysis (TESA) - identify TEs in RNA and DNA sequencing datasets.

Combined biological approaches→ transcriptomics & genomics→ to detect gene & transposon expression changes

Multiple comparisons will allow us to correlate specific TE expression changes to the molecules involved in horizontal gene transfer and serves as a cost effective platform for microgravity studies.

The Process

Will reveal the role of extreme environments:

- Activating transposons as a molecular mechanism of horizontal gene transfer
- DNA repair
- Incorporate new genetic material (foreign DNA) into the rotifer genome

Long-term Benefits & Social Impact

- Potentially breakthrough for gene therapy for diseases like cancer and/or correct inherited disorders.

Future Biotechnology

May facilitate gene therapy→circumvent current CRISPR technology→ random off-target gene alteration and on-target errors→ caused by insertion of random RNA sequences in the cytoplasm (during normal cellular activity).

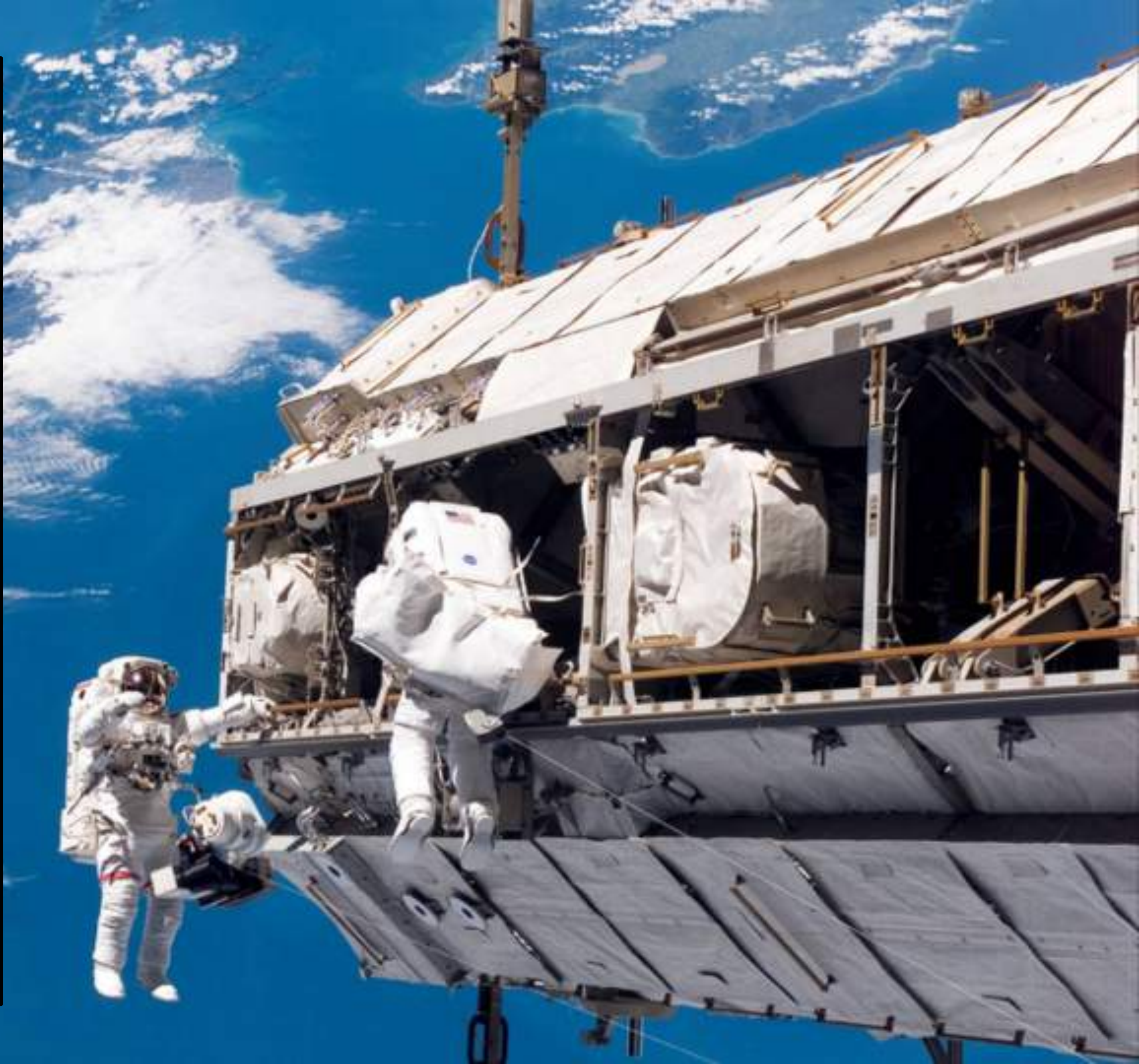
Future Direction - Potential Scientific Impact

There are genome editing tools such as CRISPR

- Current biotechnology methods for *in vivo* genetic engineering are cumbersome
- Intrinsic error yielding a low success rate

Knowledge

- The role of transposons as a molecular mechanism of DNA repair and horizontal gene transfer as an adaptive response in microgravity
- Provide a blueprint for manipulating these molecules
- The first steps to revolutionizing the next-generation genetic engineering methodologies involving horizontal gene transfer



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Significant to Spaceflight

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