Plant Cultivation for Space Exploration

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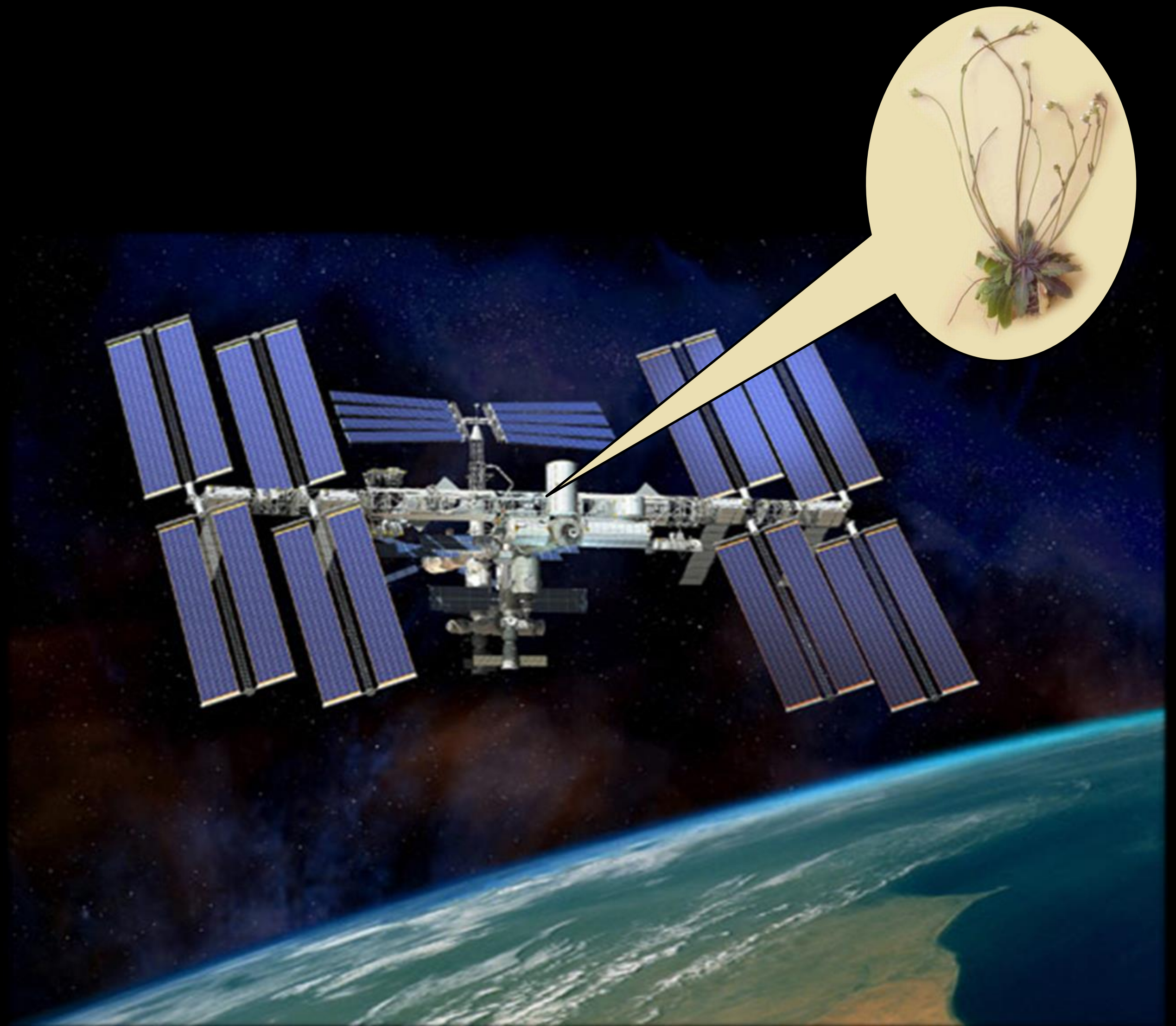
Plants in Space:

- *What do they serve for?*
- *What do we know and what do we need to learn on them?*

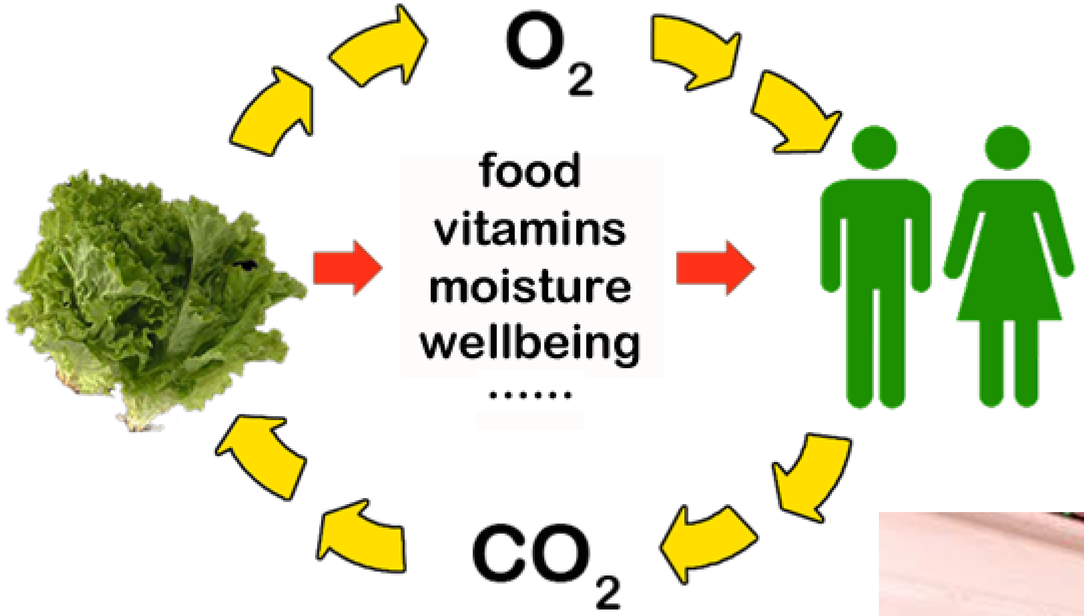
Plants in Space:

Two key Objectives:

- To contribute to make possible **HUMAN EXPLORATION OF SPACE**
- To learn essential concepts on **PLANT PHYSIOLOGY AND STRESS RESPONSE**

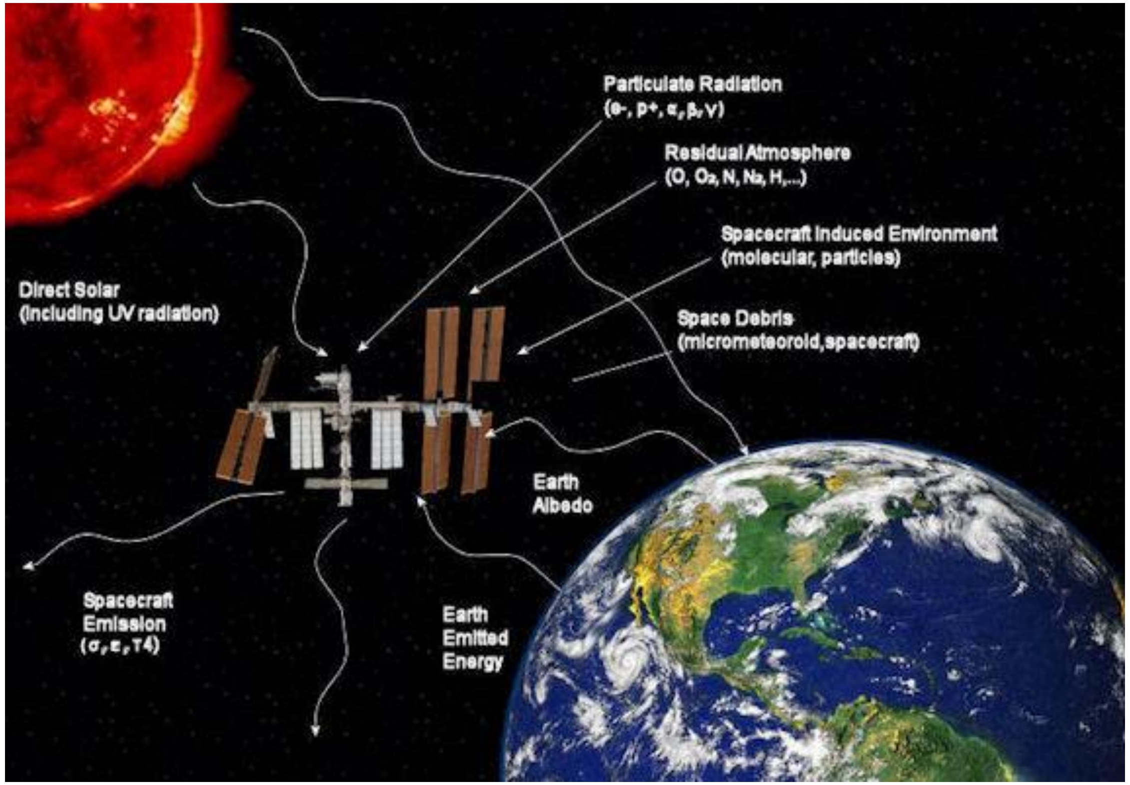


Plants are a key component to support Human Life in Space



Long-term human survival in Space (e.g. Space Exploration) requires **Life Support Systems**, in which plants occupy a key position

Space environment is very different from Earth environment



Radiation

Temperature

Magnetism

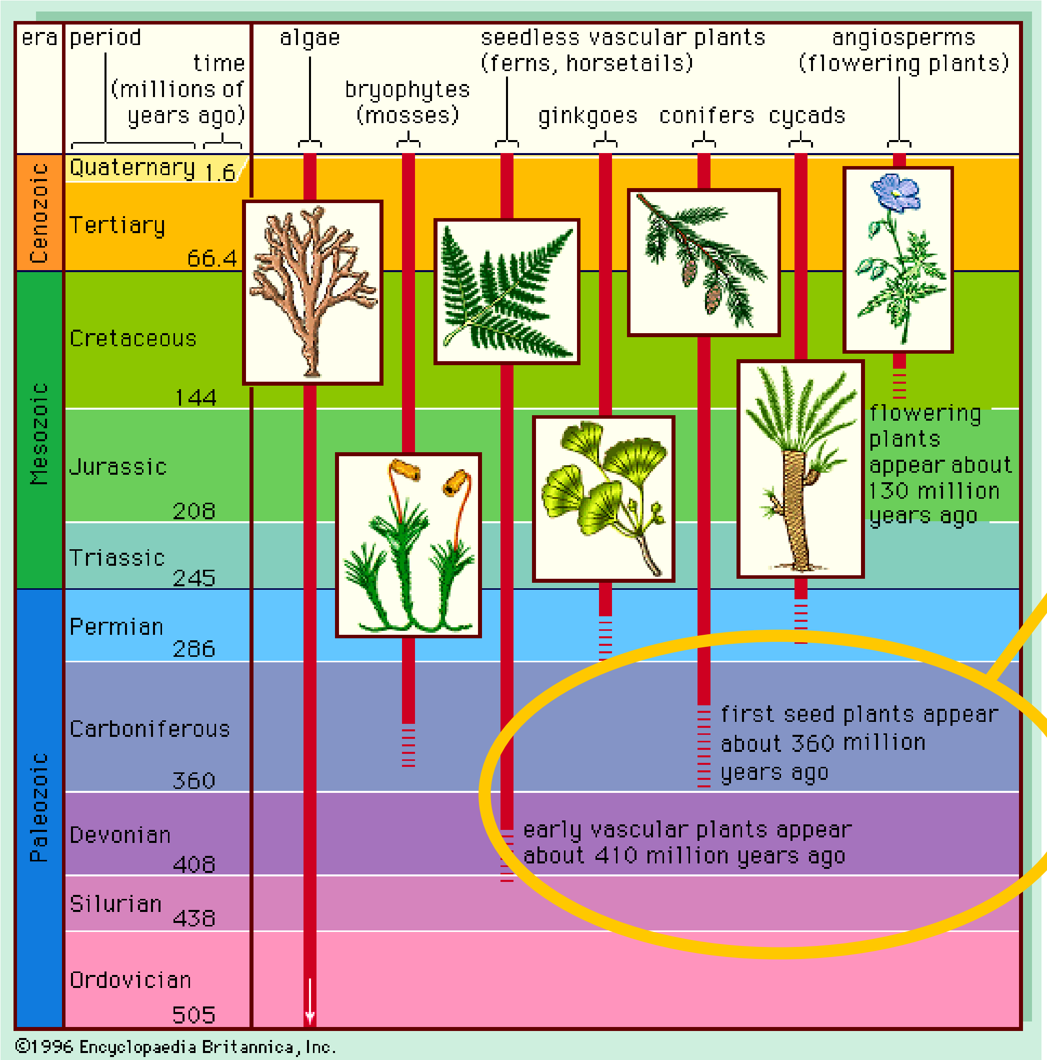
Atmosphere

GRAVITY

Gravity is a key environmental factor in Earth

- On Earth, Gravity is an all-pervading environmental factor
- Gravity has shaped our World.
- Earth History (including Biological Evolution) has occurred in the presence of Gravity.
- Many Physical Processes, affecting biochemical reactions, are caused by Gravity.

(Plant) Evolution has occurred in the presence of the EARTH ENVIRONMENT (<u>Gravity</u>, Atmosphere, etc.)



A critical step in Plant Evolution: Colonization of mainland by aquatic plants:

- Avoid desiccation.
- Develop a rigid body to resist the force of **gravity**.
- Orient growth according to **gravity** vector.

Zero-Gravity (microgravity) is a key environmental factor in space

- Objects orbiting the Earth are in continuous "free fall" in order to keep constant the distance of the orbit to the Earth.
- The result is "zero-gravity", or "weightlessness", or "microgravity" ($<10^{-3}g$).

Moon gravity = $0.17g$
Mars gravity = $0.38g$
(according to Newton's Law)

What is the effect of altered gravity on living beings? (in particular, on plants)

Contents

Gravitropism

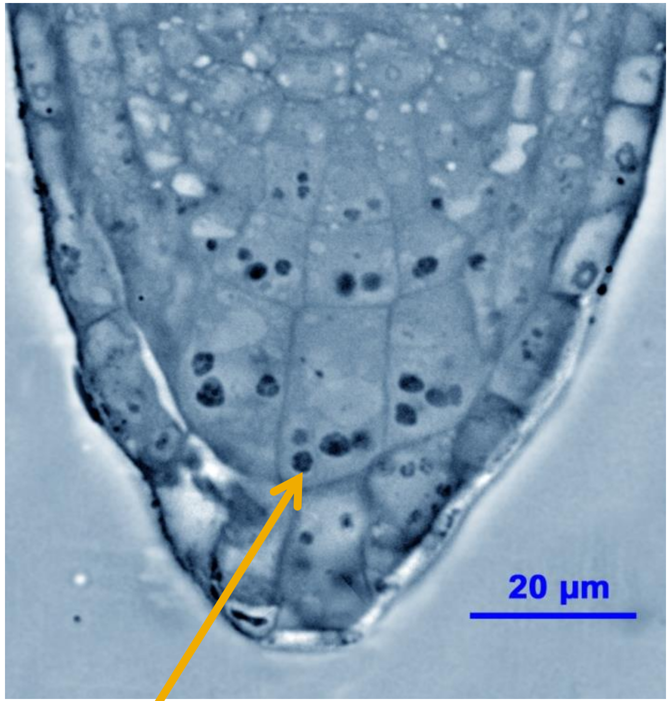
GRAVITROPISM is the most conspicuous effect of Gravity on Plant Growth

- Plant Growth is oriented according to the gravity vector. Roots grow downwards and stems grow upwards.

- This is crucial for plant survival: roots anchor the plant to the soil and extract water and nutrients from it; leaves should be maximally exposed to sunlight for the most efficient performance of photosynthesis.

- When a plant is placed horizontally, the root and stem bend and reorient their growth according to the gravity vector.

Root gravitropic response depends on statolith movements



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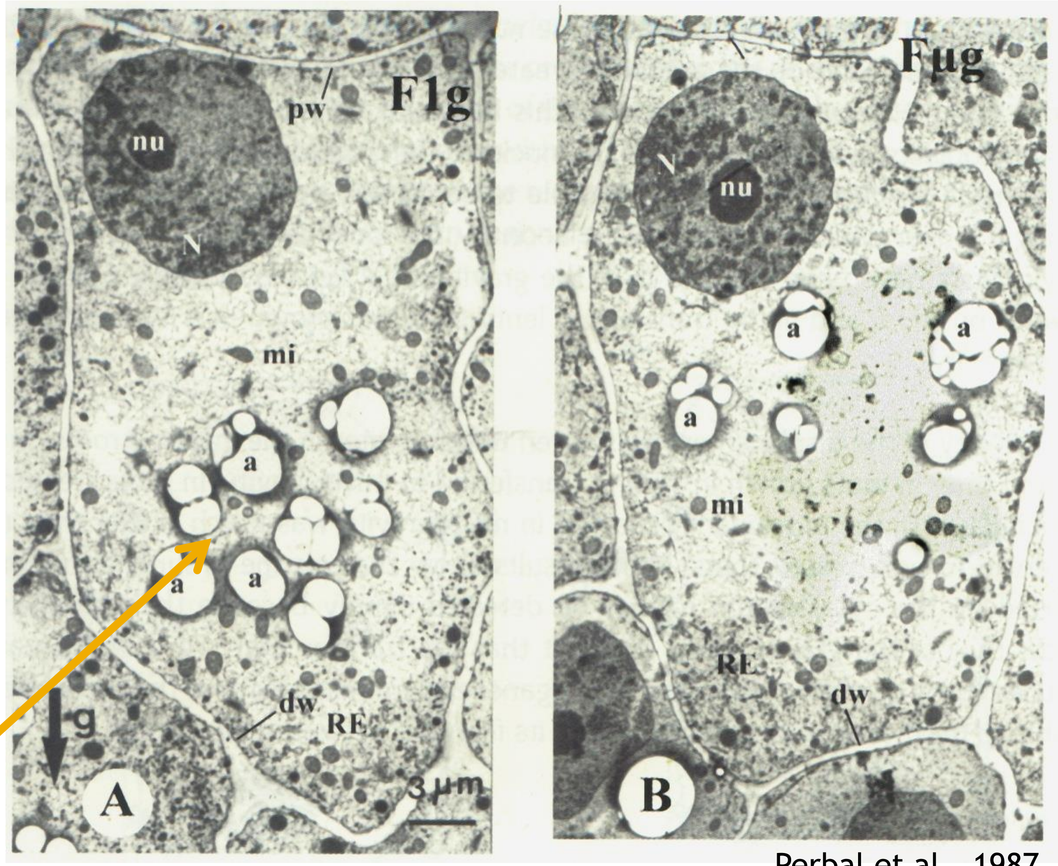
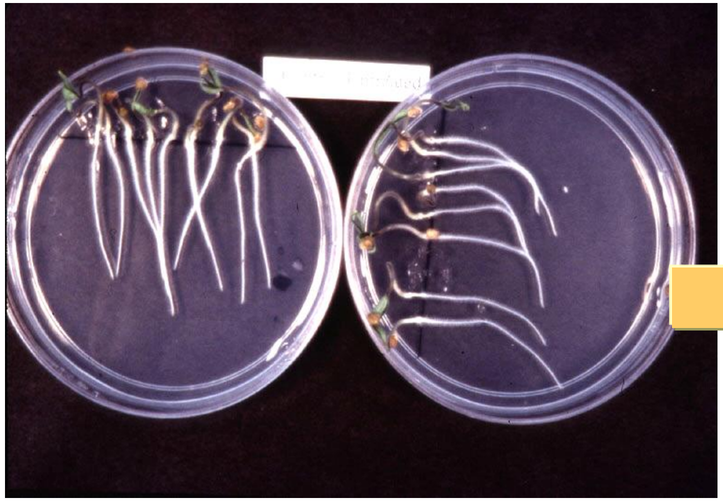


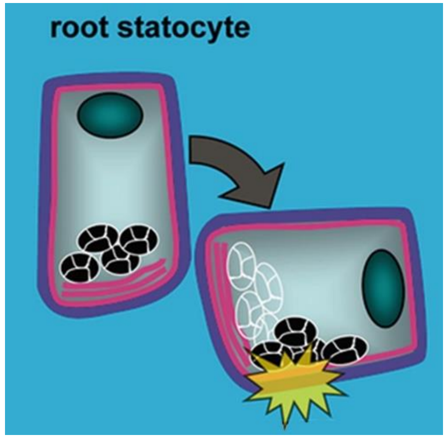
Perbal et al., 1987

Statoliths are starch-containing amyloplasts located in columella cells of the root cap

A change in the magnitude or in the direction of the gravity vector leads to statolith re-distribution

Root gravitropic response involves cell membranes, calcium channels and auxin flux





Gravity Alteration
↓
Statocyte Movement
↓
Pressure on Membranes (ER and Plasma Membrane)
↓
Activation of Calcium Ion Channels
↓
Re-localization of efflux auxin carriers (PIN proteins)
↓
Auxin differential secretion
↓
Differential cell elongation in a specific zone of the root
↓
Root Bending

Gravitropic response ultimately depends on the transport of the phytohormone auxin throughout the root

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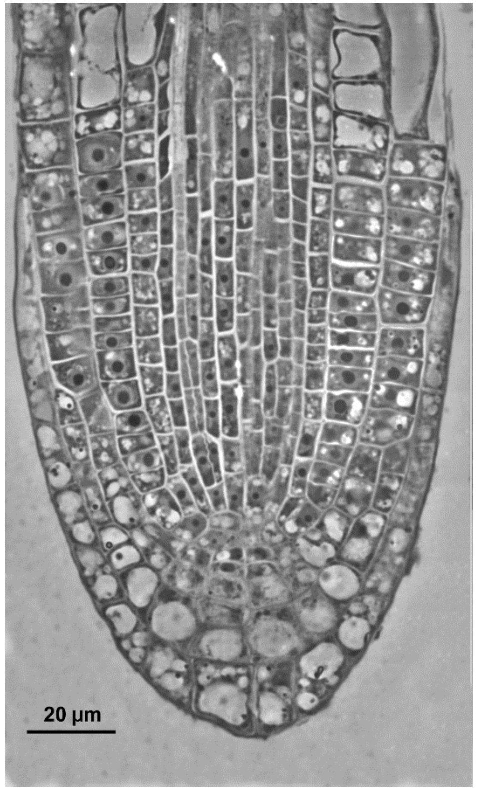
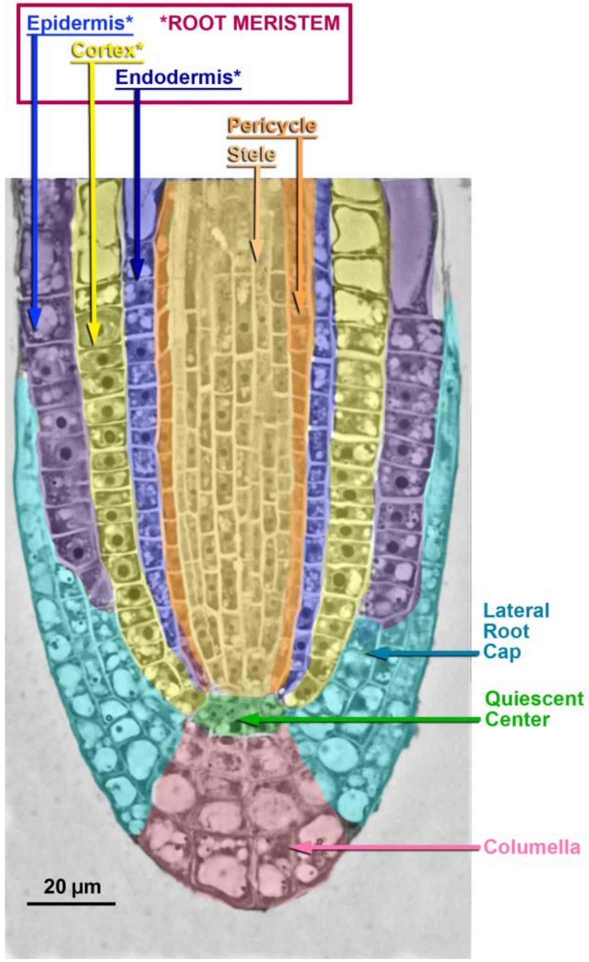
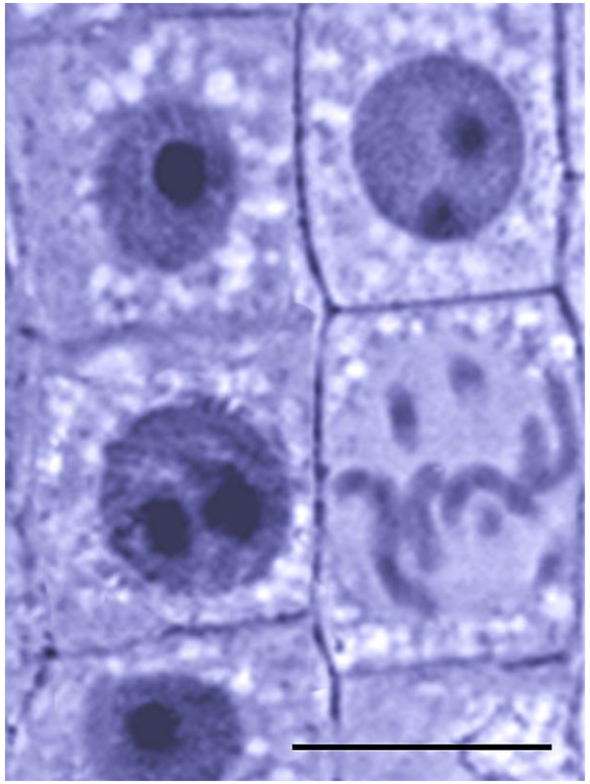
The biological problem we are investigating:



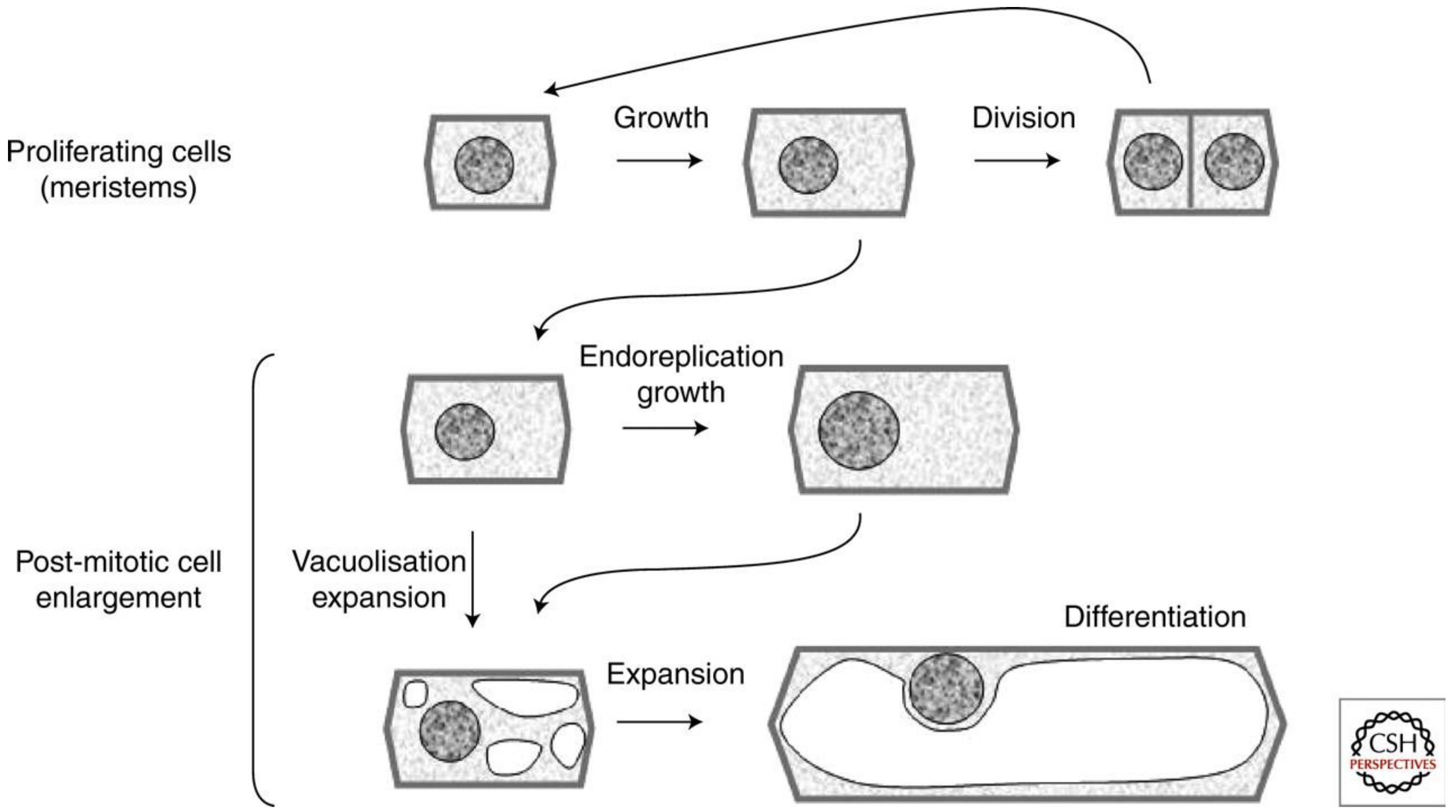




The Root Meristem

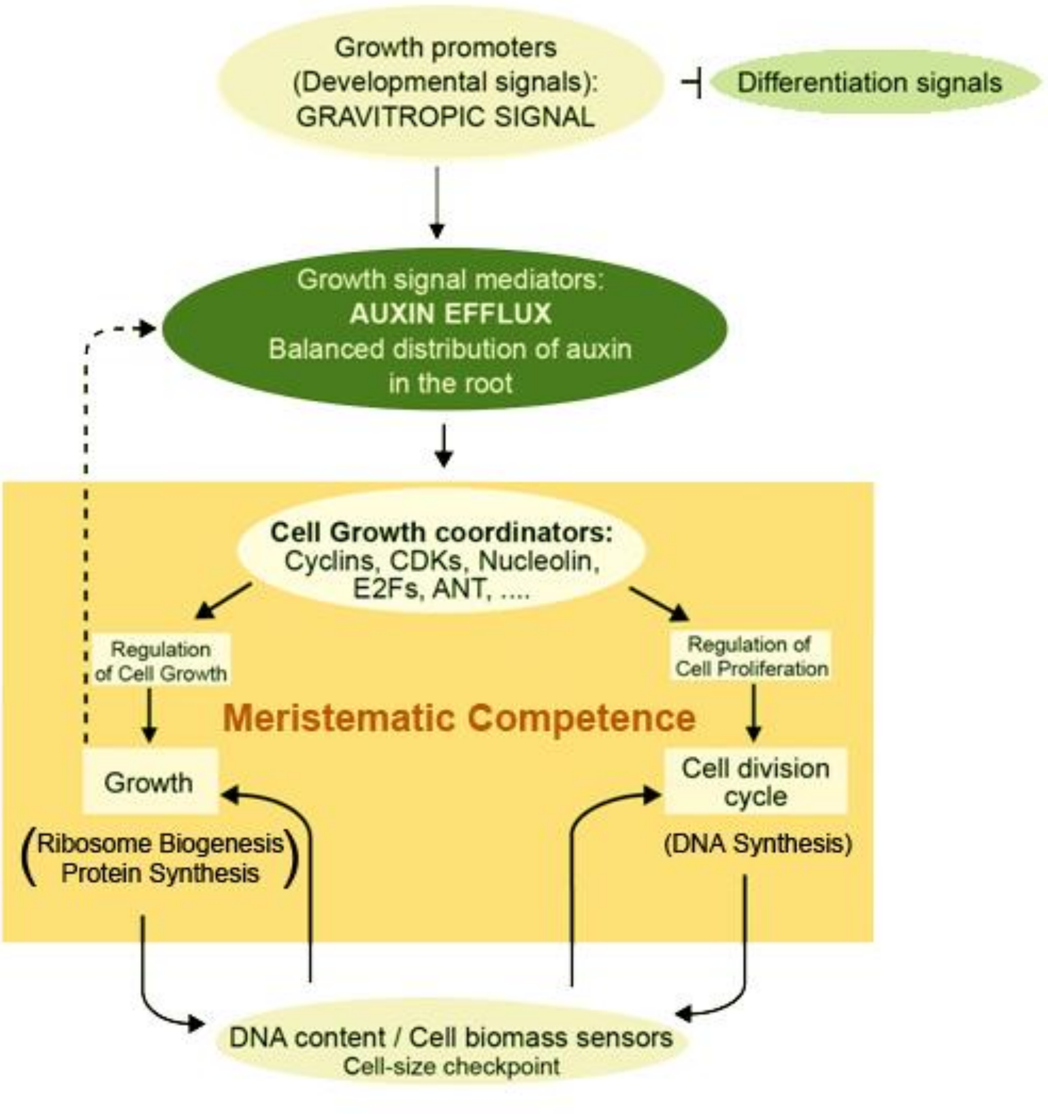
The biological problem we are investigating:

Plant Growth and Development depend on the balance between
CELL PROLIFERATION and **CELL DIFFERENTIATION** in ***Meristems***



Perrot-Rechenmann (2010)

Cell Proliferation / Cell Growth, Auxin, Gravity



GRAVITROPIC SIGNALS transduced from GRAVITY SENSORS exert an influence on Auxin transport and distribution in the root

AUXIN plays a key role in the coordination of Cell Growth and Cell Proliferation in Meristematic Cells

MERISTEMATIC COMPETENCE
is the balanced equilibrium and coordination of cell growth and cell proliferation that exists in meristematic cells

Medina and Herranz, 2010

Contents

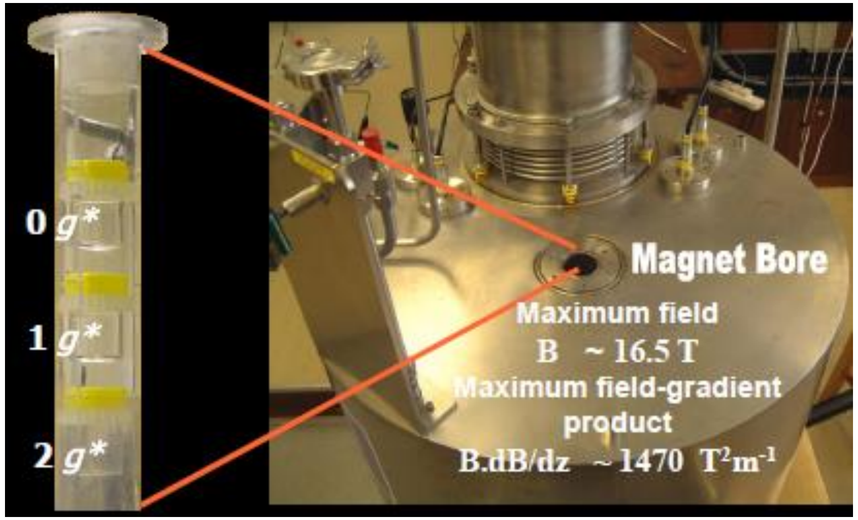
The International Space Station (ISS)

The most advanced facility constructed up to now for microgravity research

Ground-Based Facilities

Microgravity Simulation:

Clinostat



Magnetic Levitation

Random Positioning Machine (RPM)

Hipergravity:

Large Diameter Centrifuge (LDC)

- **Provide flexibility, easy access and a reasonable reliability.**
- **Offer different gravity levels (micro-*g*, hyper-*g*, fractional *g*).**

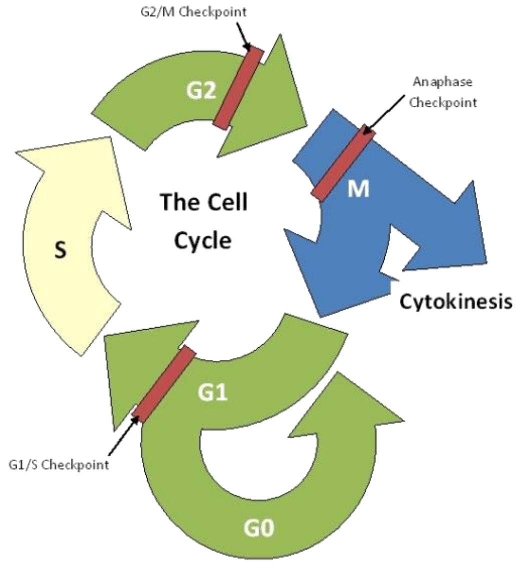
The “ROOT” Experiment

- “Cervantes” (Spanish Soyuz) Mission to the International Space Station. October 2003.
- The Spanish ESA astronaut Pedro Duque performed 24 scientific experiments in the course of a 10-day stay in ISS.

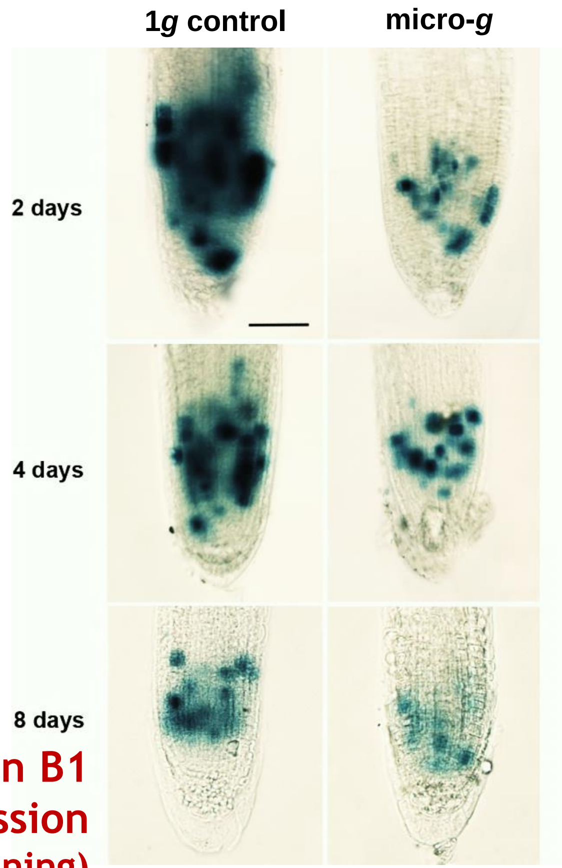
The “ROOT” experiment was the First European Experiment on Plant Biology in the ISS

Under Gravitational Stress, Cell Cycle Regulation and Auxin Polar Transport are altered

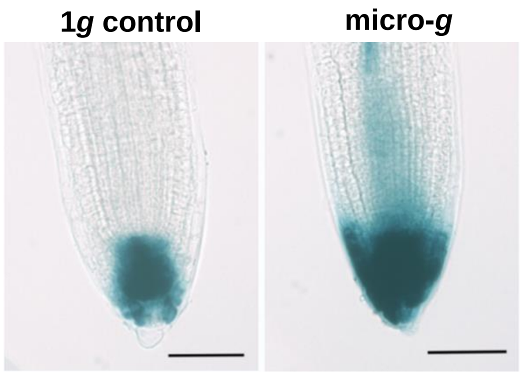
Experiments in real and simulated microgravity:



The expression of some genes which regulate the **Cell Division Cycle** is altered.



Cyclin B1 expression
(CYCB1::GUS staining)

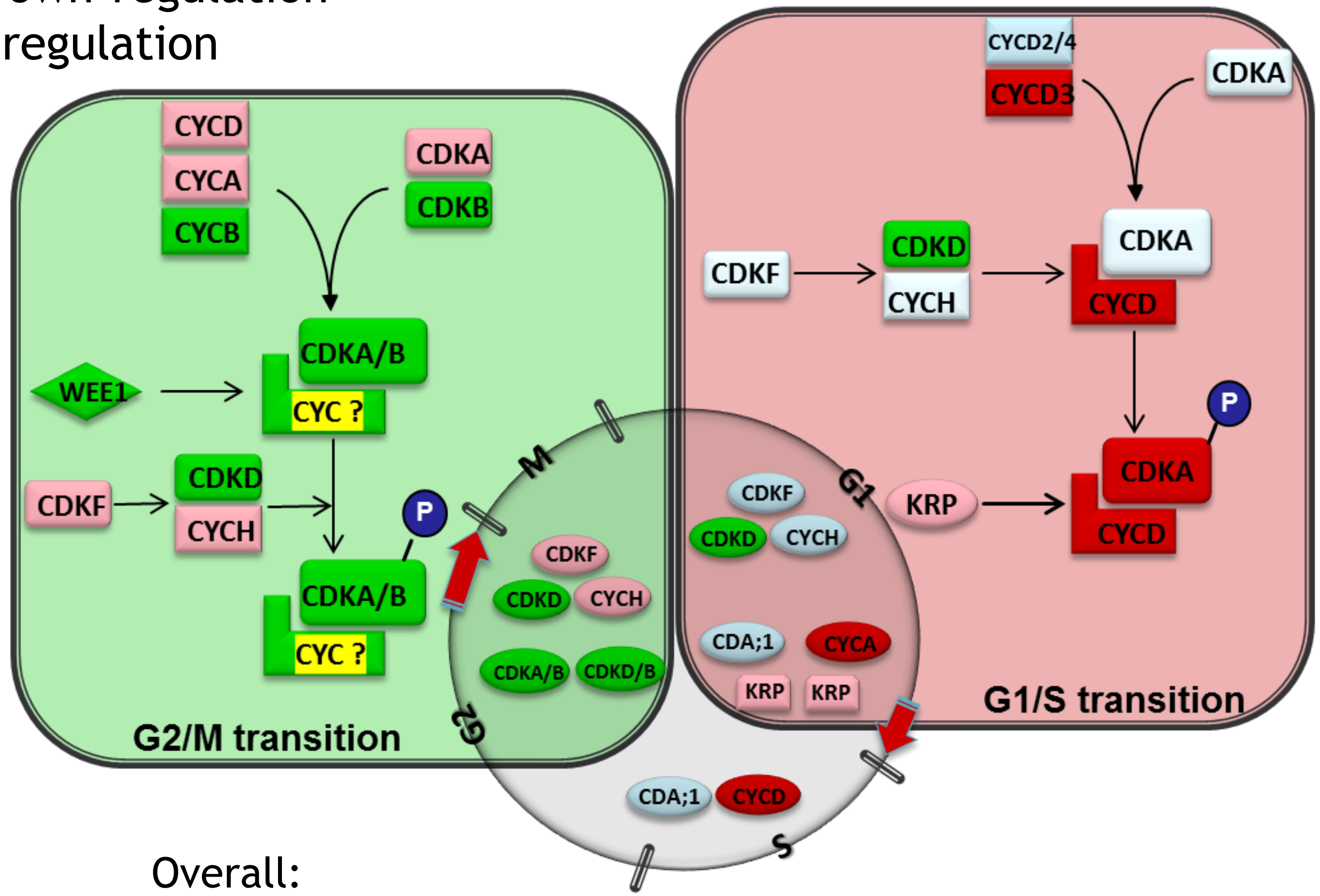


Auxin distribution
(DR5::GUS staining)

The pattern of **auxin distribution** in the root tip corresponds to a **partial inhibition of Auxin Polar Transport**.

Alterations in the expression of Cell Cycle regulatory genes in microgravity, detected by DNA chip microarray technique

Green: Down-regulation
Red: Up-regulation



Results obtained on
in vitro cell cultures:

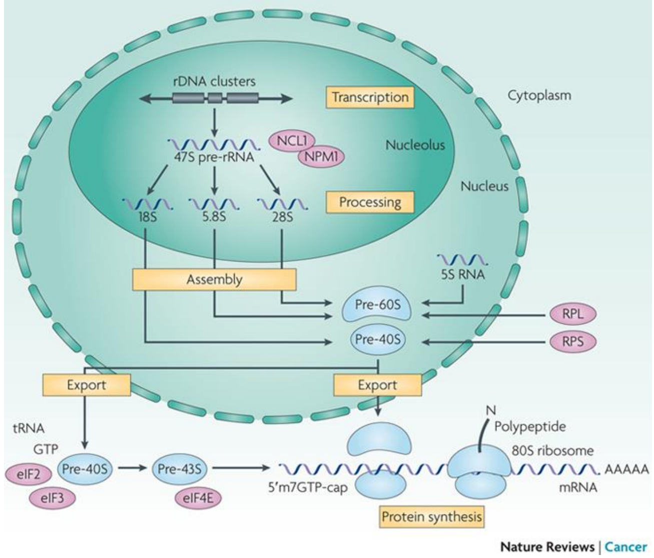
This is a Cellular Response

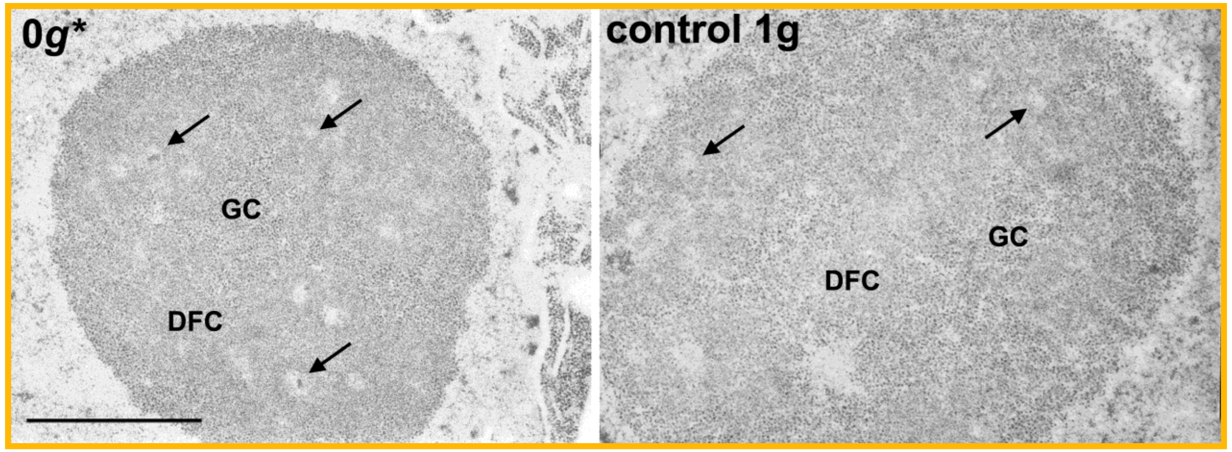
Overall:

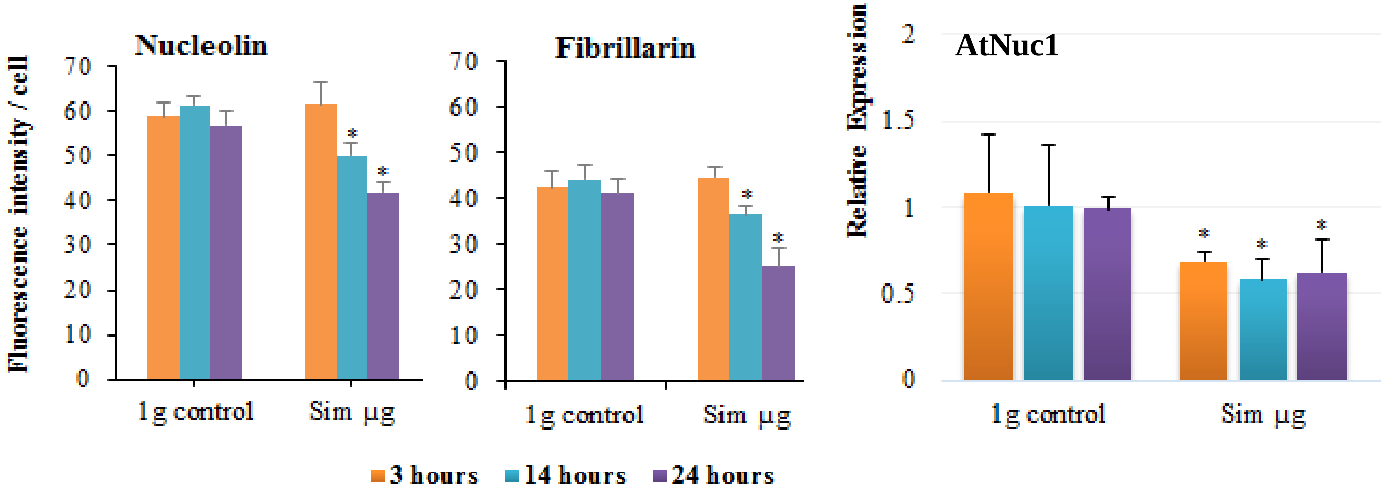
- G2/M transition is down-regulated: G2 phase is shorter and mitosis occurs earlier.
- G1/S transition is up-regulated: G1 phase is slightly longer.

The final result is <u>cell cycle acceleration</u>
(increase of the cell proliferation rate)

Cell Growth (protein synthesis) is reduced in microgravity







Alteration of environmental gravity is a **major stress condition** for the plant, detectable at the molecular, cellular and macroscopic level, which induces the triggering of **survival mechanisms**.

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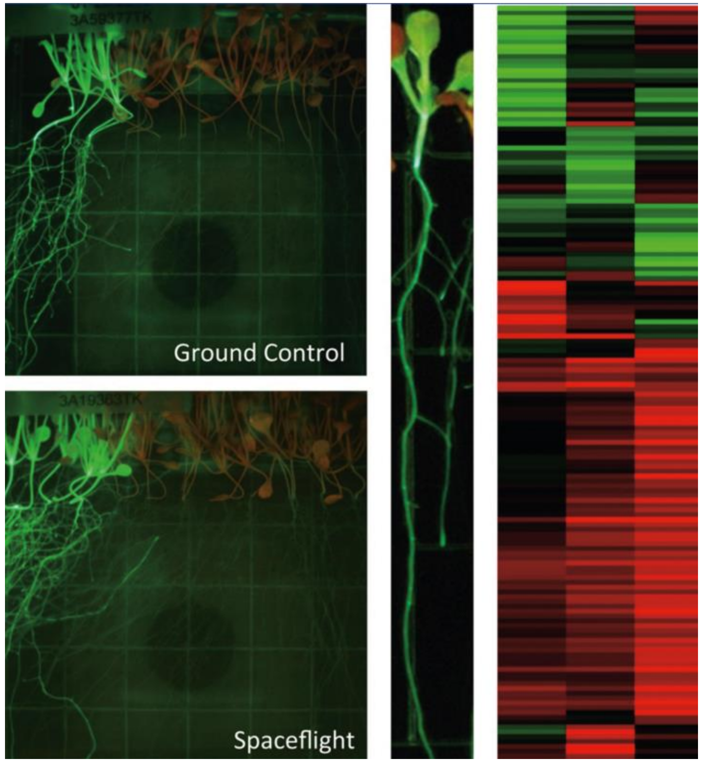
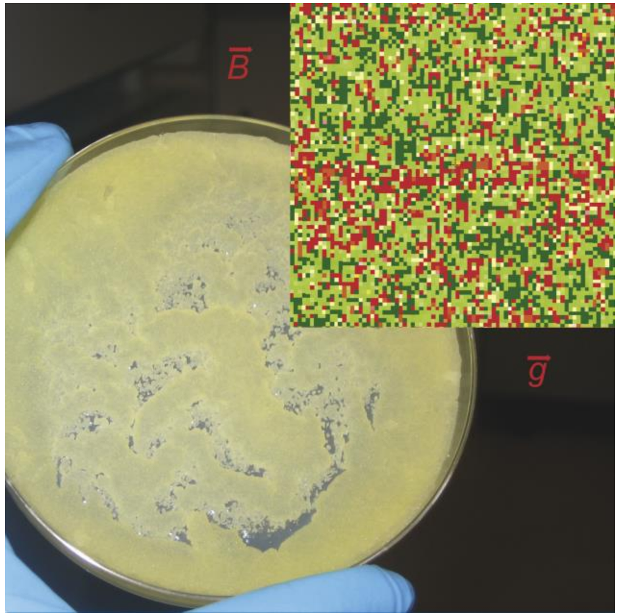
Microgravity causes Gene Reprogramming in Plants

Transcriptomic Studies:

- Seedlings, Plant Organs, Whole Plants, Cell Cultures
- Real or Simulated Microgravity

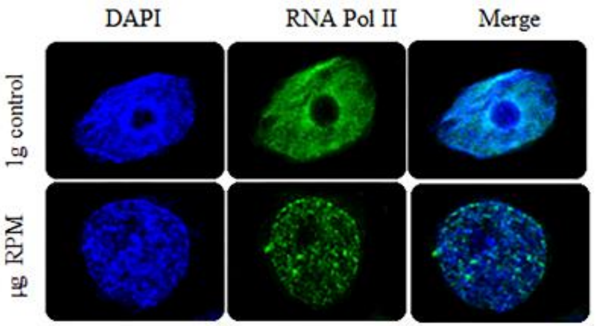




Major Functional Categories Affected:

- General Abiotic Stress Response (Heat Shock).
- Cell Wall Remodeling.
- Oxidative Stress.
- Genes of Unknown Function.

No Specific Genes of Response to Gravity Alteration have been found



Epigenetic Mechanisms (Chromatin Condensation) have been found to be involved in the response to Microgravity

Contents

Plants survive in space

- Plants may eventually overcome weightlessness and other adverse factors of the space environment.

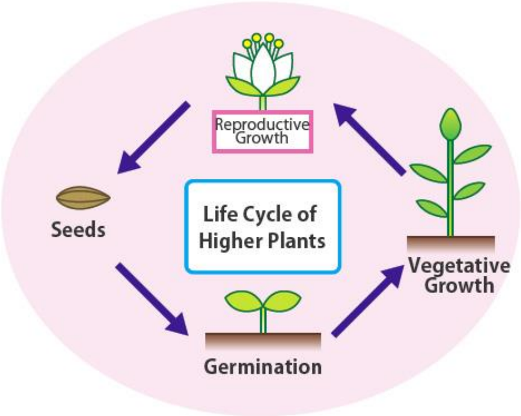


Figure 1. The Life Cycle of Higher Plants

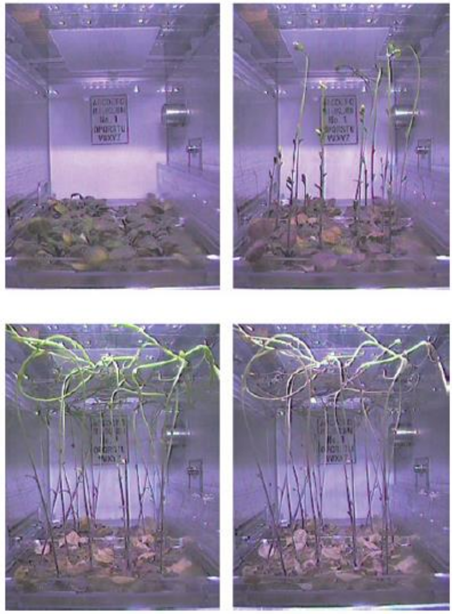


Figure 3. Growth of Arabidopsis Thaliana Growth in the PEU for from 0 to 42 Days

- A full Plant Life Cycle (seed-to-seed) has been achieved in ISS.
- “Space Seed” Experiment, carried out in the Japanese “Kibo” module in 2009
- NASA “Veggie” experiment: an adult edible plant was grown in ISS and snacked by astronauts, in 2015

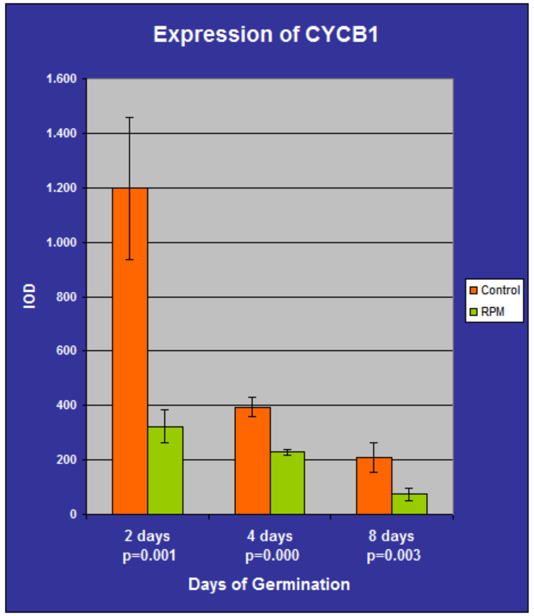
Keyword is ADAPTATION

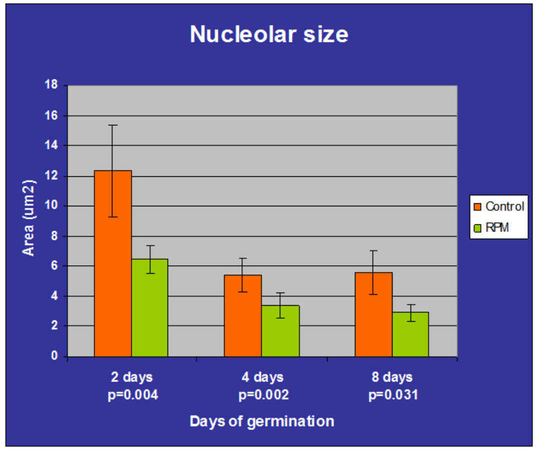
- Between the severe damage observed in early development and the robust and viable adult plants there must be a process of **Adaptation** o **Acclimation**, by which the plant counteracts damages, restores fundamental parameters and develops with apparent normality.

- The mechanisms of this process are unknown.

- Our sequential experiments have shown a certain attenuation of the effects in more developed seedlings.

- This fact is not exclusive for plants: in Drosophila fruit flies grown in microgravity, the apparent paradox of *the existence of strong cellular and molecular alterations, compatible with an apparently normal development until the adult stage* was reported (Roberto Marco et al., 2003).





This is one of the most exciting challenges of the current Space Biology

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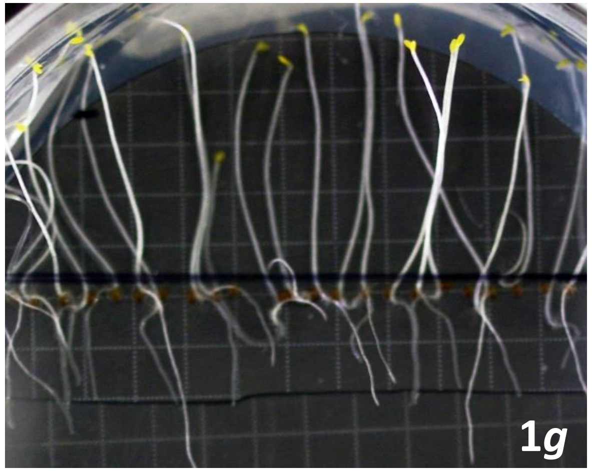
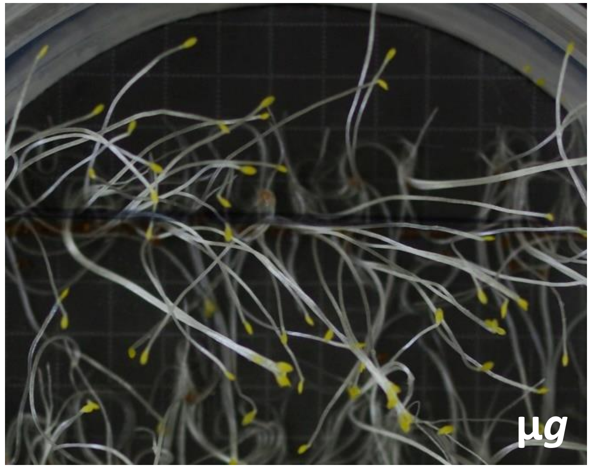
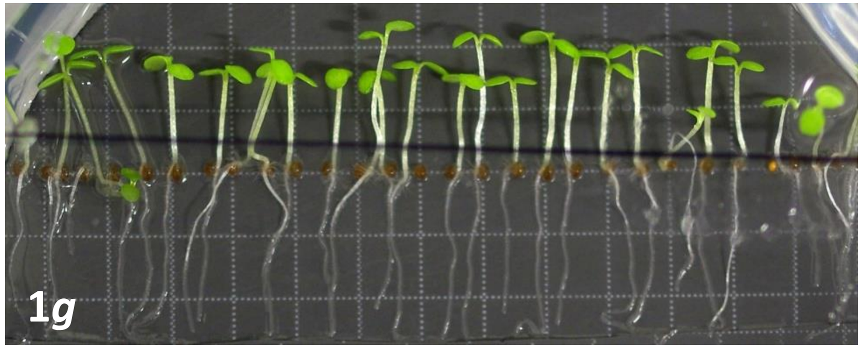
Light may restore plant growth orientation in weightlessness



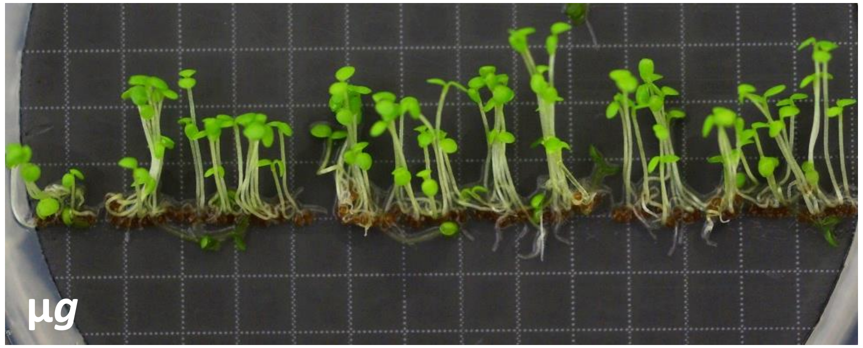


With light:

The position of the light source is the only reference for growth (Phototropism):



The response is different in roots and shoots



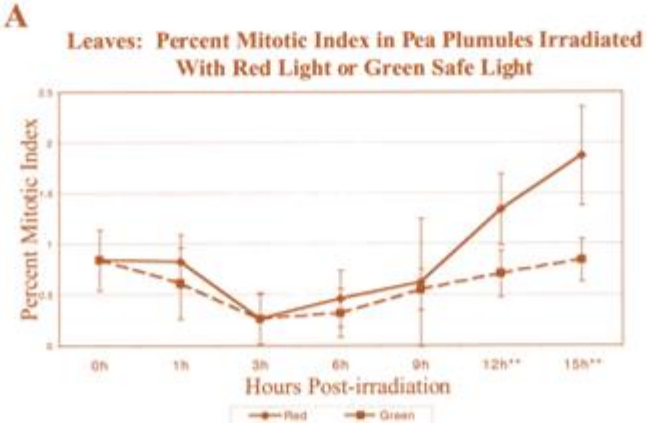
Without light:

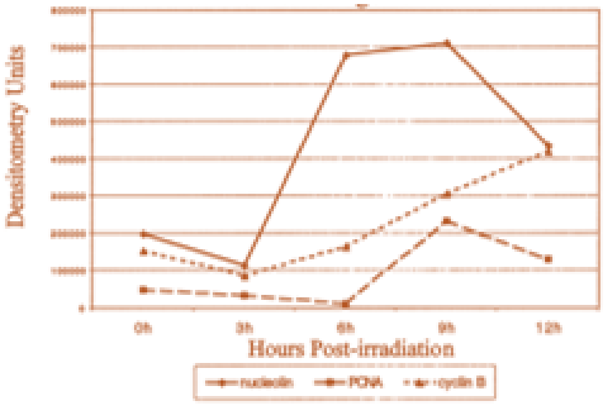
Seedlings lack of any orientation in their growth

May light play a role in the mechanisms of adaptation to space environment?

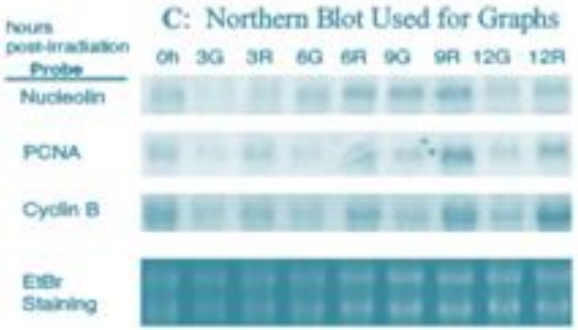
PREVIOUS DATA: Red Light irradiation activates cell growth and cell proliferation

Red light irradiation enhances cell proliferation (mitotic index)





Red light irradiation increases phosphorylation of nuclear proteins

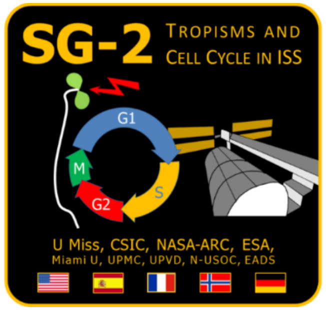


Red light irradiation induces expression of nucleolin and cell cycle genes

- Datta, N., Hardison, L. K. & Roux, S. J. (1986) Plant Physiol., 82, 681-684.
- Reichler, S. A., Balk, J., Brown, M. E., Woodruff, K., Clark, G. B. & Roux, S. J. (2001) Plant Physiol., 125, 339-350.
- Tong, C. G., Reichler, S. A., Blumenthal, S., Balk, J., Hsieh, H. L. & Roux, S. J. (1997) Plant Physiol., 114, 643-652.

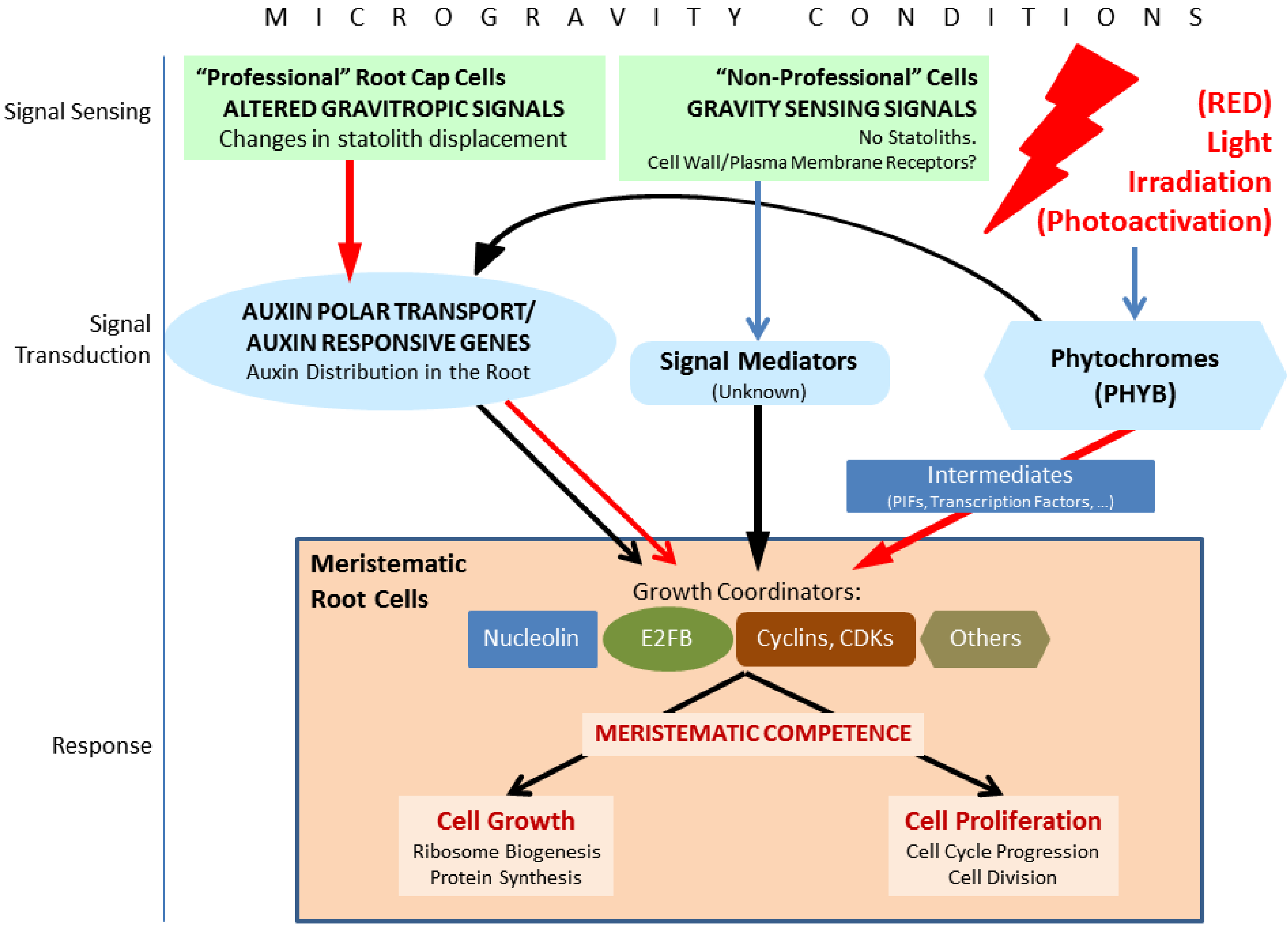
Seedling Growth:
A complex multinational project in ISS

Interaction between light and gravity sensing and its effects on cell growth and proliferation in plant seedlings grown in space



PIs: Prof. **John Z. Kiss**, UNCG, USA & Dr. **F. Javier Medina**, CIB-CSIC, Spain

WORK HYPOTHESIS: Light and gravity stimuli may synergistically regulate cell growth and proliferation in the root meristem

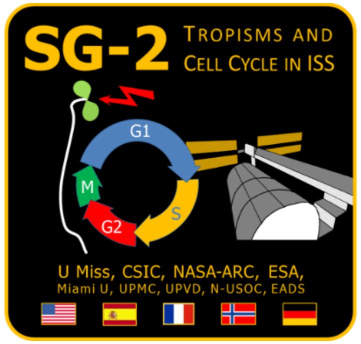


Seedling Growth consists of three parts

Seedling Growth-1 (SG1)

Launch SpX-2	01 March 2013
Experiment ISS	22 March – 24 May 2013
Recovery SpX-3	18 May 2014

- Analysis of phototropism in **microgravity** conditions.
- Influence of phototropic alteration on cell growth and proliferation.



Seedling Growth-2 (SG2)

Launch SpX-4	21 Sept 2014
Experiment ISS	01 Nov – 15 Dec 2014
Recovery SpX-5	11 Feb 2015

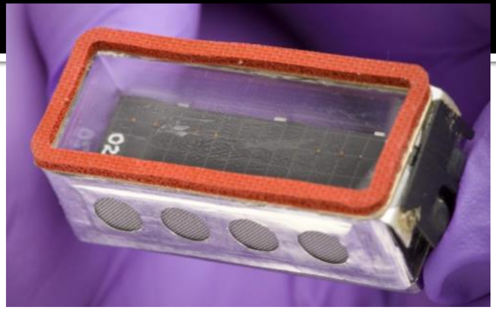
- Analysis of phototropism in conditions of **fractional gravity (Moon, Mars).**
- Effects of red light photoactivation on cell cycle and ribosome biogenesis and their regulation by auxin. Studies of gene expression.

Seedling Growth-3 (SG3)

Launch SpX-11	3 June 2017
Experiment ISS	13 June – 25 June 2017
Recovery SpX-11	4 July 2017

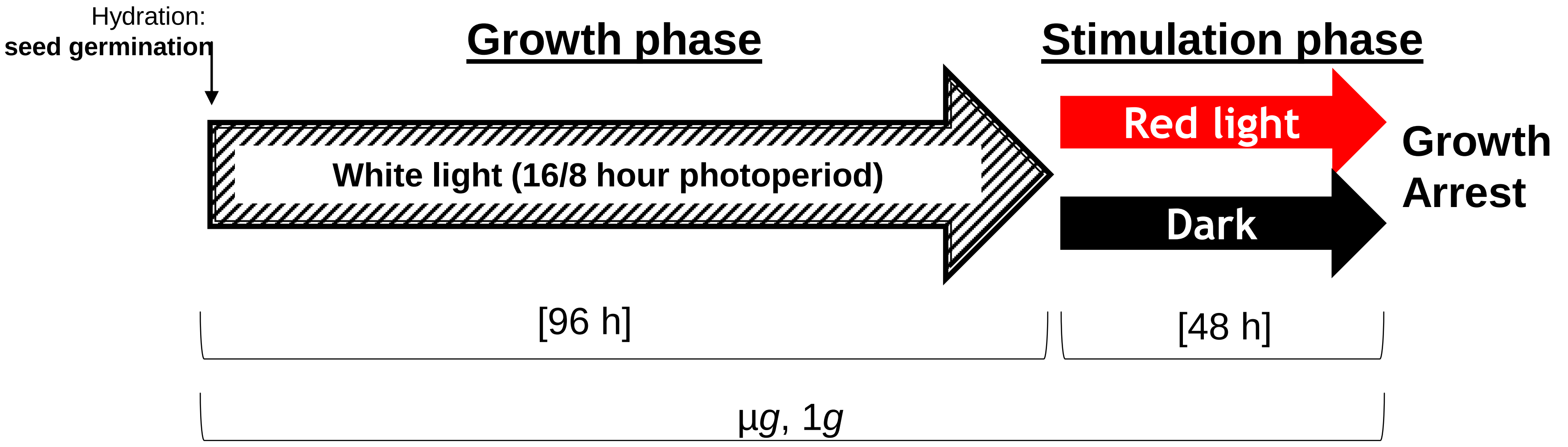
- Effects of red light photoactivation on cell cycle and ribosome biogenesis and their regulation by auxin. Studies of cell biology by microscopic observation.
- Studies of gene expression and cell biology in conditions of **fractional gravity (Mars).**

The Space Experiment



Arabidopsis thaliana ecotype "Columbia"
Genotypes: Wild Type (Col0)
Three mutants for auxin transport:
(eir1.1, tir1, aux1.7)
Two mutants for the nucleolar protein nucleolin:
(nuc1, nuc2)

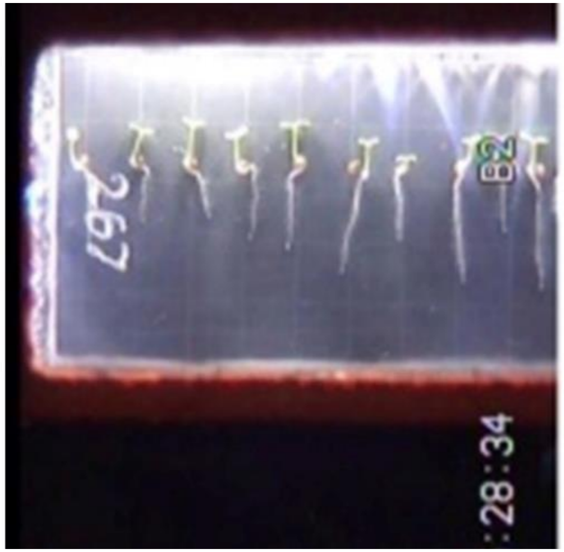
Growth Procedure:



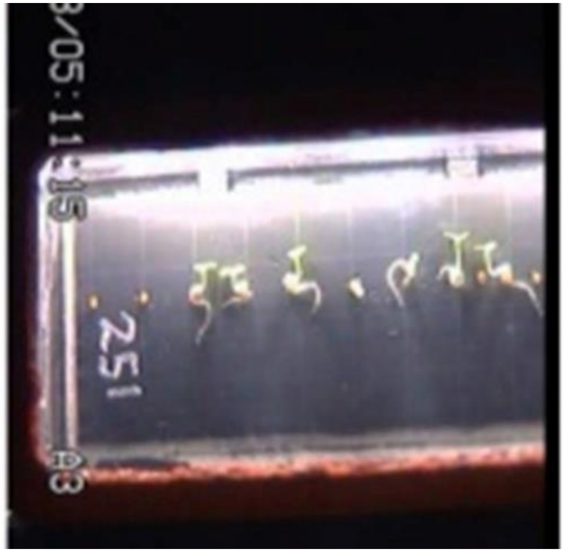
Conditions:
- Red light 1*g*
- Red light micro-*g*
- Darkness 1*g*
- Darkness micro-*g*

Sample preservation was:
- by DEEP FREEZING (-80°C), in MELPHI
- by CHEMICAL FIXATION (PFA, GA), in FIXBOX

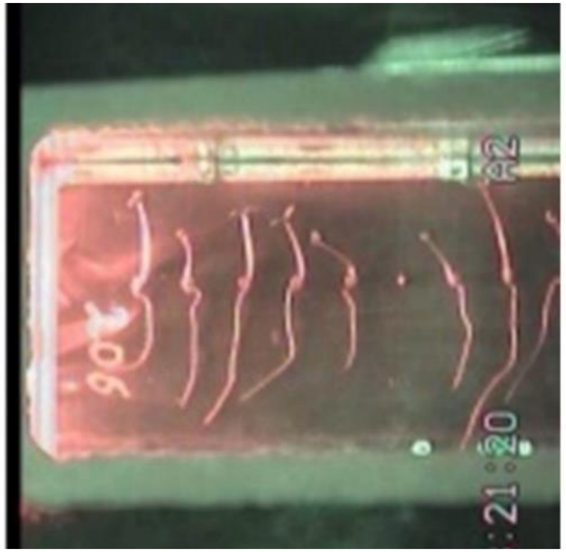
Samples grown in space



1g - White light

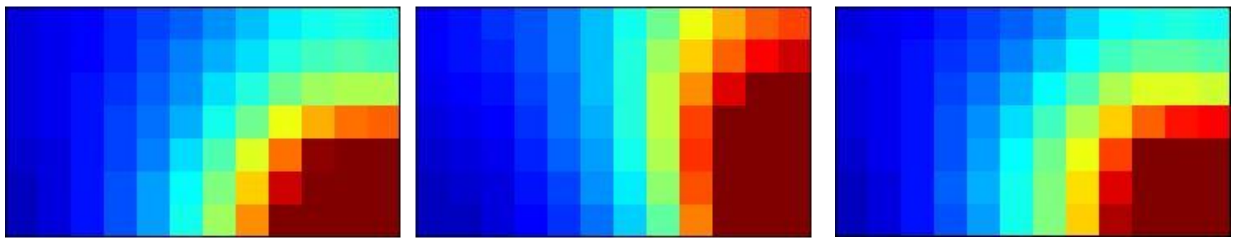


µg - White light



(1g+µg) - Red light

The pattern of Gene Expression is restored in microgravity after red light photoactivation

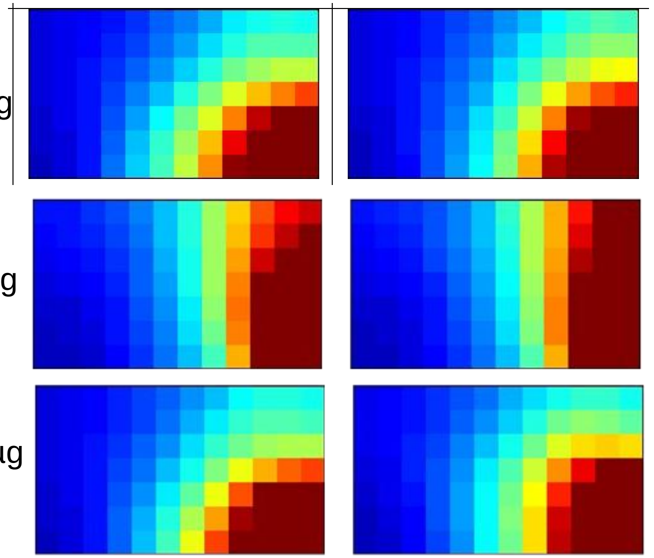


Global Transcriptomic Study (RNAseq)

Visual analysis of changes using the GEDI tool

Microgravity induces differences in gene expression. These differences are counteracted by red light treatment.

No significant differences appear between genotypes

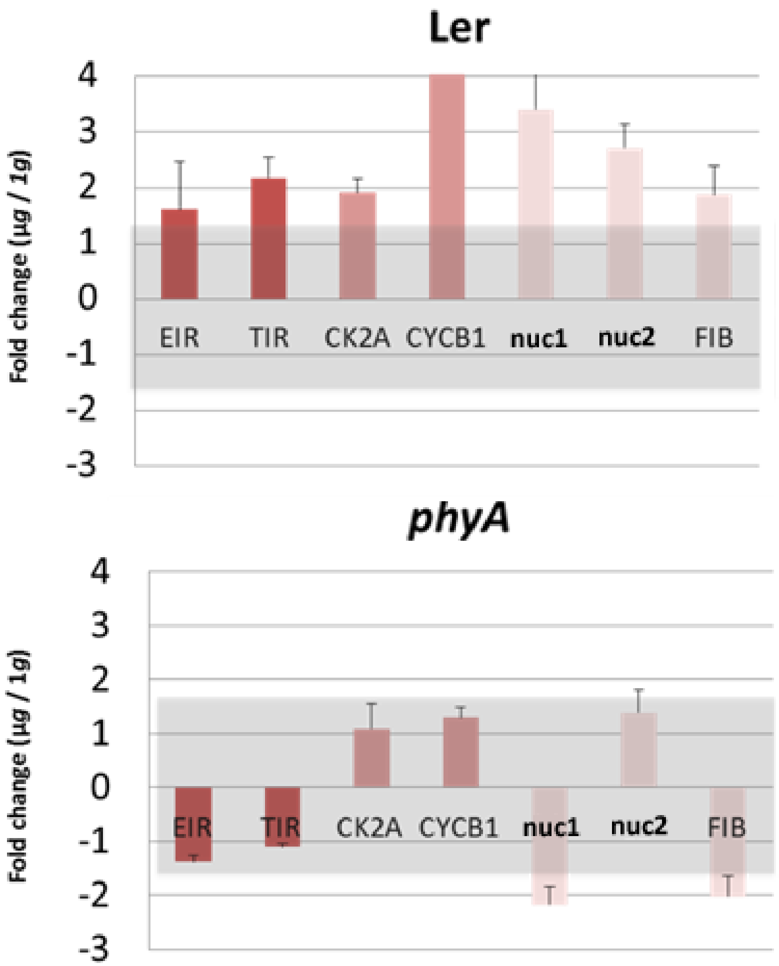


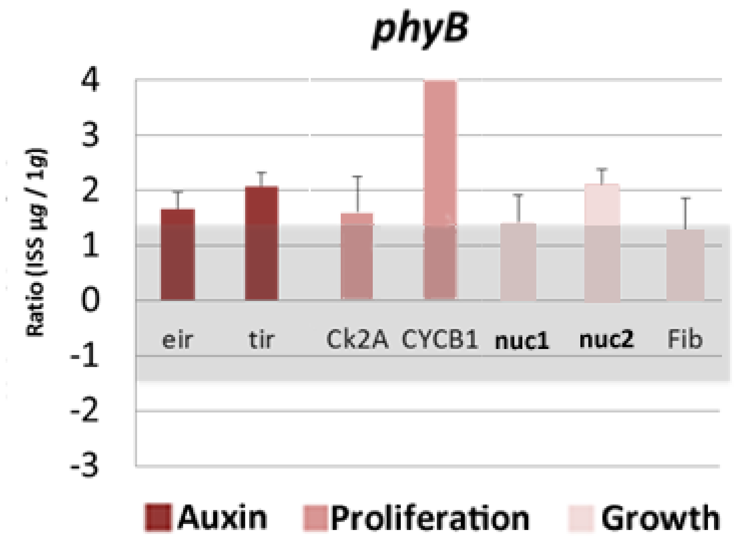
Seedlings grown in microgravity (6 days) and irradiated with red light (2 days)

Transcriptomic study on cell proliferation: Red light restores meristematic competence

Seedlings grown in microgravity (6 days) and irradiated with red light (2 days)

Differential expression (qPCR; µg *vs* 1*g*) of genes of auxin transport, cell cycle (proliferation) and ribosome biogenesis (growth) in seedlings of wild type (Ler) and two phytochrome mutants (*phyA* and *phyB*)

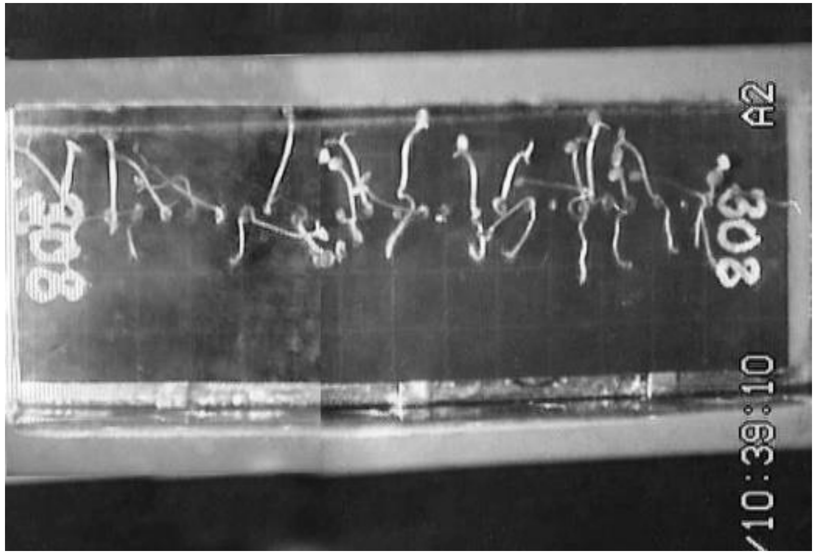




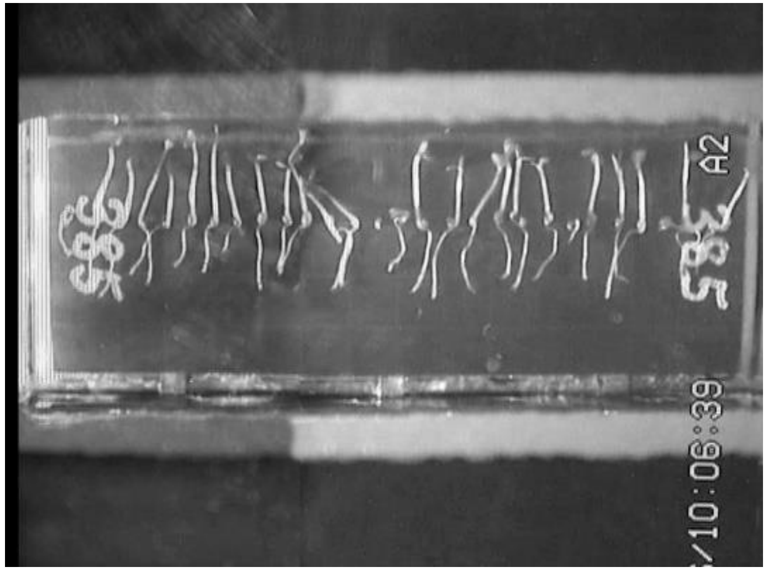
- Genes of cell proliferation and cell growth are coordinately upregulated.
- Auxin transport genes are upregulated.
- The results of the *phyA* mutant strongly diverge from Ler and *phyB*.

What happens under Mars Gravity?

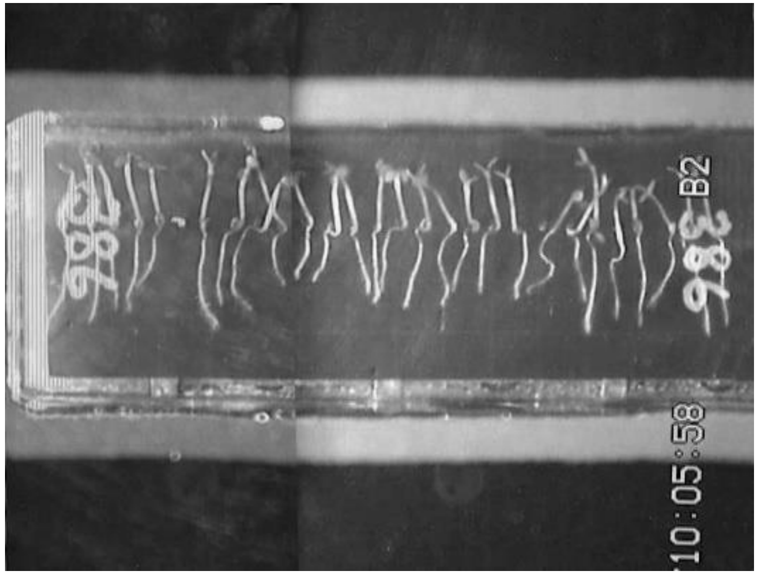
- Roots are capable of detecting Mars gravity (0,38*g*)



Micro-g



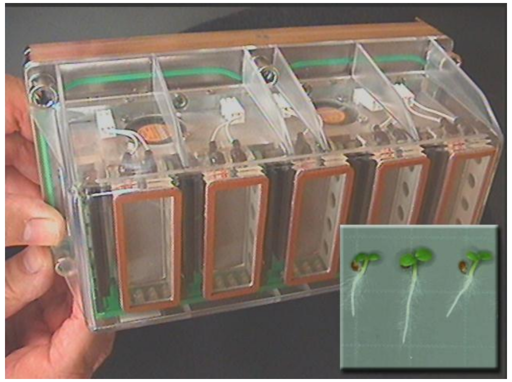
0,3g
(Mars gravity)

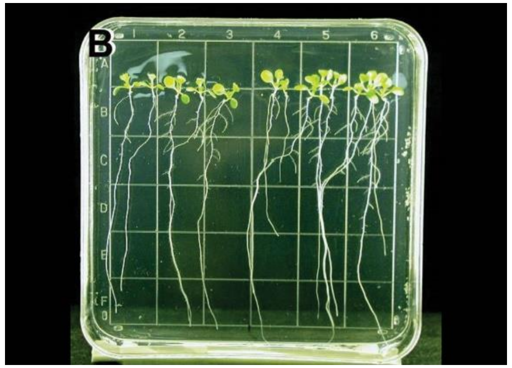


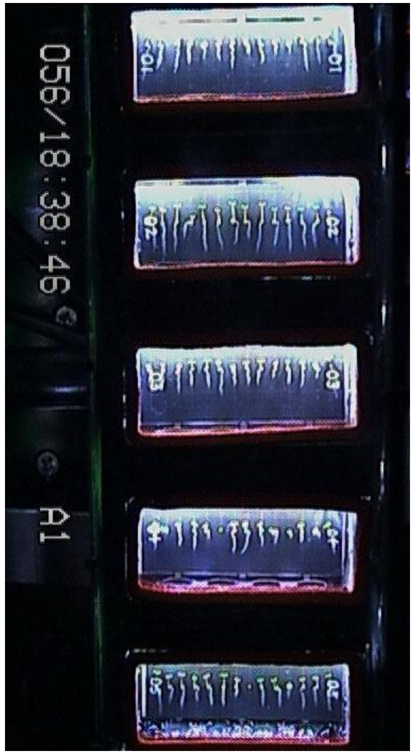
1g
(Earth gravity)

Contents

From Model Plants to Crop Plants







Arabidopsis thaliana

- Small size.
- Easy culture.
- Well known (physiology, genetics,...).
- Availability of mutants.

Crop plants (tomato, vegetables, salad plants, ...)

- Highly variable for many parameters.
- Large size.
- Little physiological and genetic knowledge.
- No culture facilities.

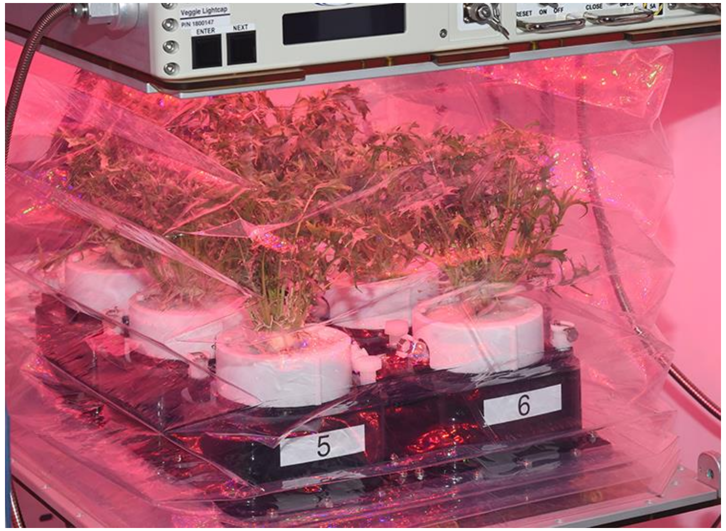
The problem of watering space plants

On Earth, the plant sits in soil within the container. When the caretaker of the plant adds water, it seeps down to the roots and then the excess water flows out of the hole on the bottom of the pot.

- In space, nothing stays put inside its container.
- The water in a planter would not flow downward, but continuously saturate—a no-no for most plants.

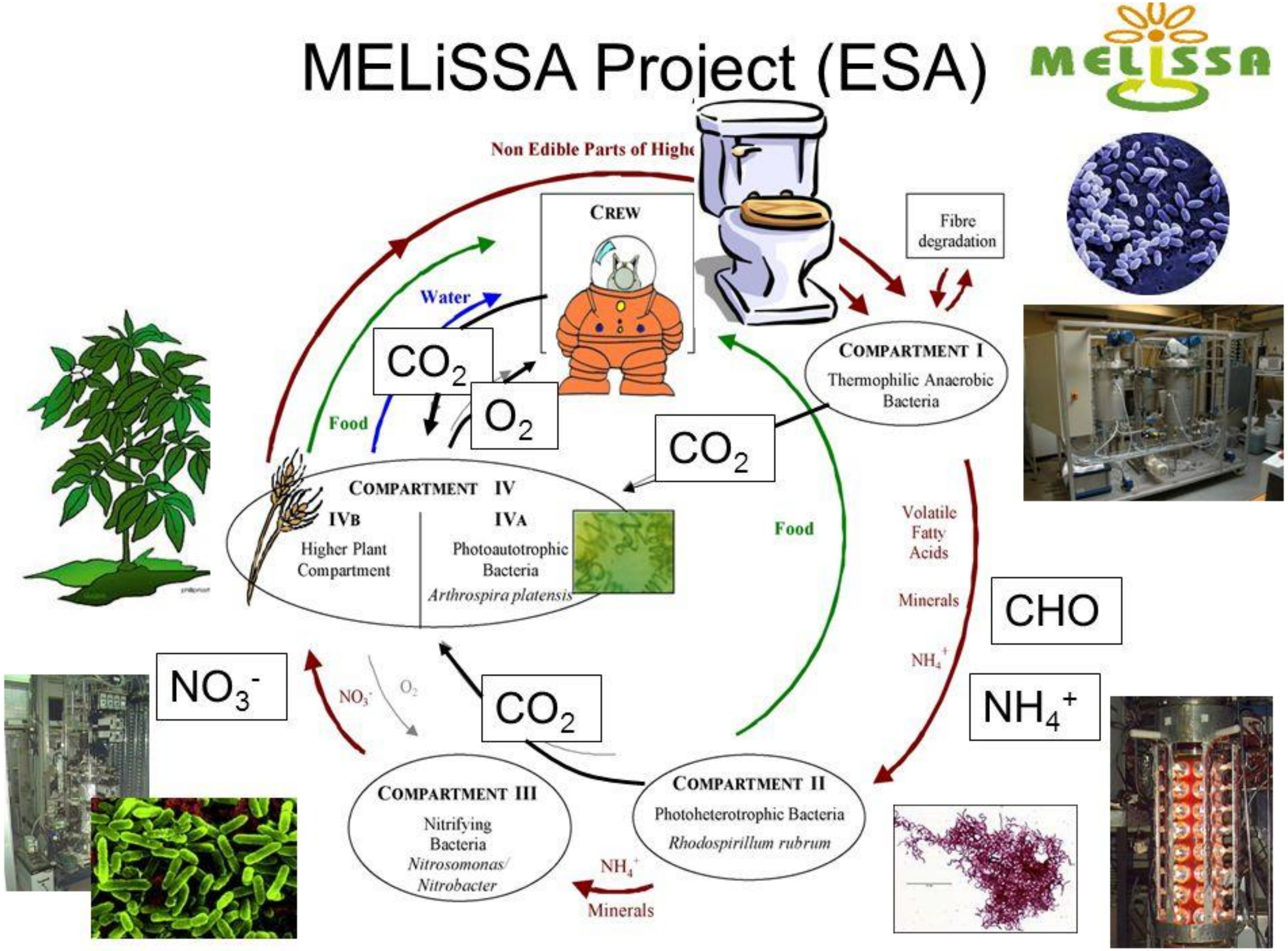
Two technological solutions implemented by NASA

“pillows”



PONDs

Testing BLLSs in Space / Microgravity



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ESA: Jason Hatton (HSF-USL, ESTEC)

Funding

esa

NASA

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