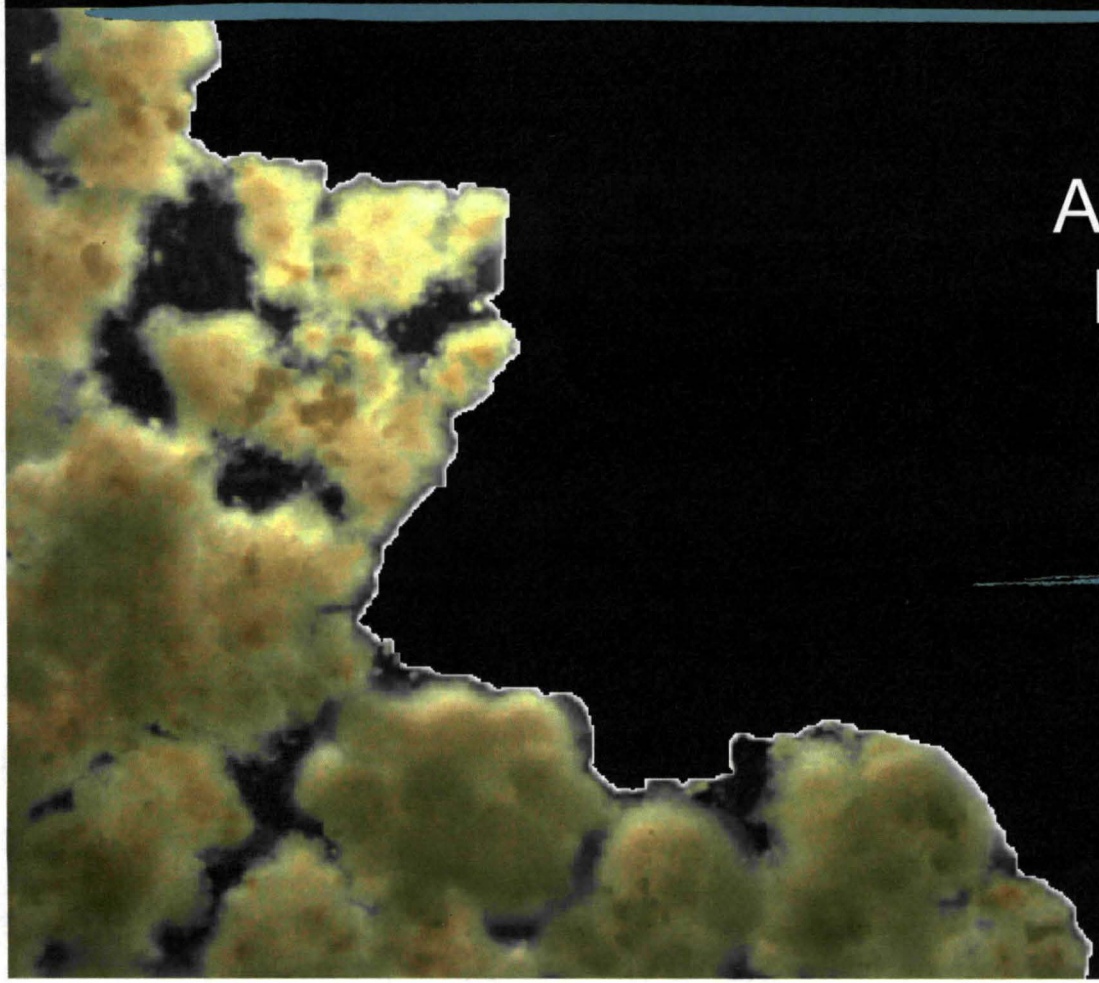
Cell Signaling in Undifferentiated Cells - Perceiving the Spaceflight Environment without Specialized Tissues



Anna-Lisa Paul - PI
Robert Ferl - CoPI

UF | UNIVERSITY of FLORIDA

Background
Hypothesis
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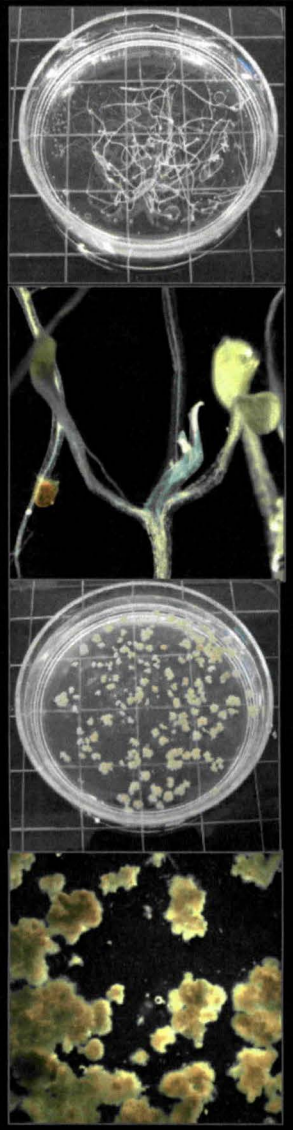
Importance and reason for ISS
Expected results and how they will advance the field
Earth benefits

Background - What we learned from BRIC-16

Arabidopsis etiolated seedlings and Arabidopsis tissue culture



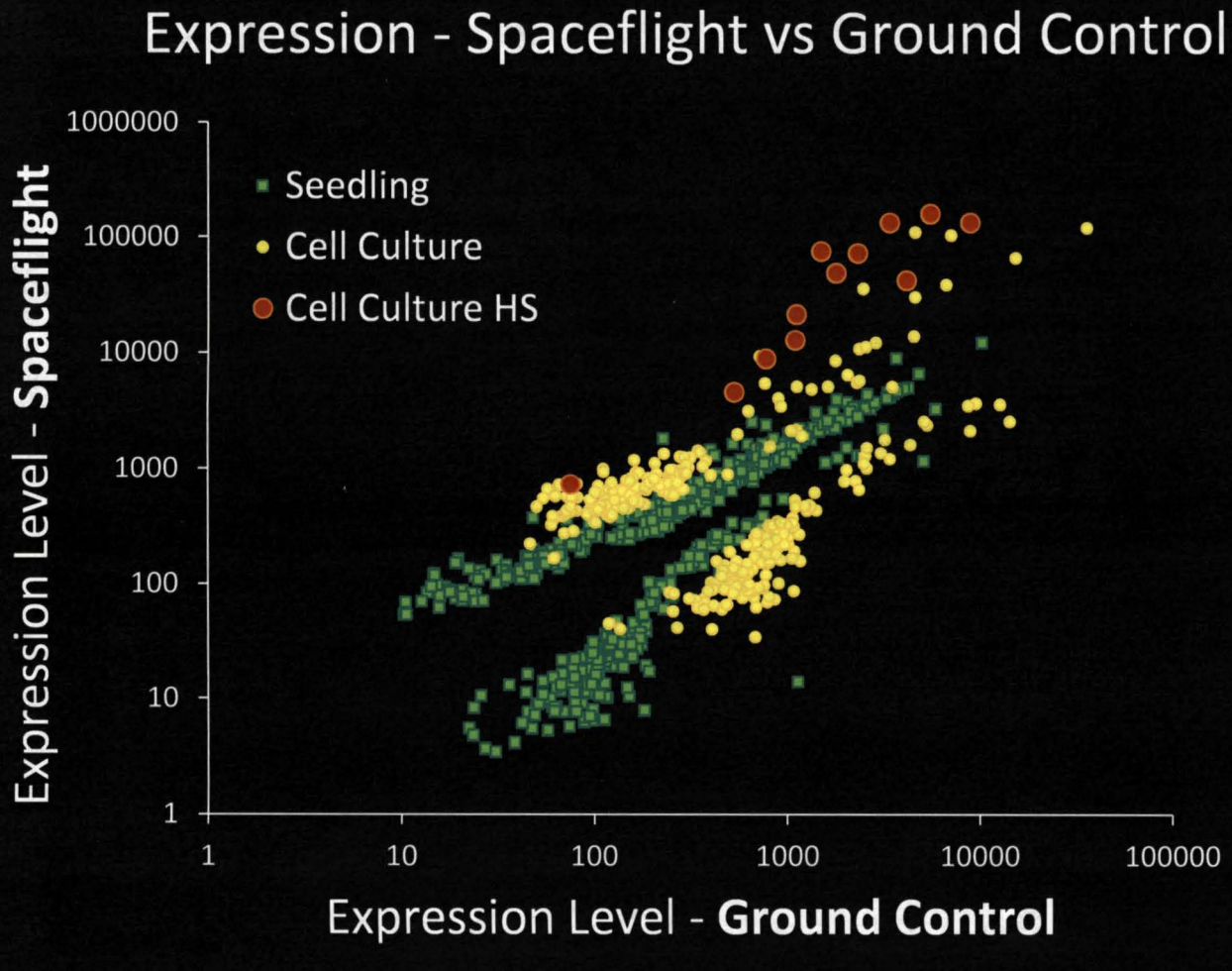
- Compare and contrast the gene expression responses within two forms of Arabidopsis:
  - whole, etiolated seedlings and,
  - undifferentiated cells in culture
  - 6 replicates each

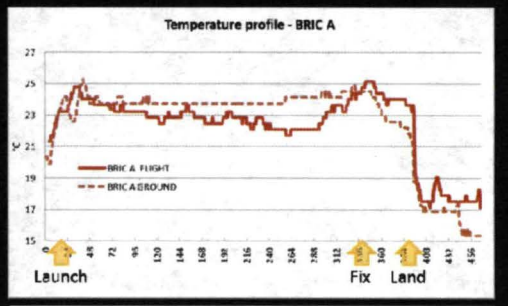
- Two complementary types of genome-wide gene expression profiling
  - Replicated DNA microarray analyses - genome-wide patterns of gene expression
  - RNA deep sequencing (RNASeq) –

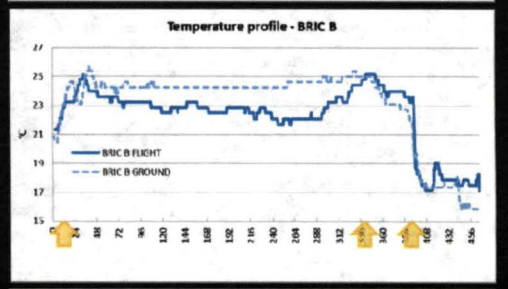
    massively parallel sequencing platforms for genome-wide quantification of transcript abundance over wide dynamic range of expression

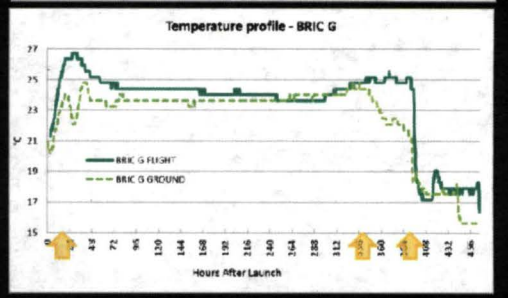
Gene Expression patterns differ between Seedlings and Undifferentiated Cells:

Background









But there were no temperature issues on orbit that would normally induce this gene set.

Conclusions for BRIC-16, and where this takes us

- The spaceflight environment requires adaptive changes that are both governed and displayed by alterations in gene expression.
- Undifferentiated cells can detect and respond to the spaceflight environment in the absence of the specialized tissues or structures known to detect gravity in plants.
- The nature of the response of seedlings and cell cultures is radically different, and many of the highly induced genes in cell cultures are characteristic of chaperone induction responses (heat shock proteins and other gene products that deal with unfolded proteins).

This discovery begs the question; *why would a suite of genes more commonly associated with heat shock and other protein unfolding stresses be induced on orbit, and why only in the cell cultures*?

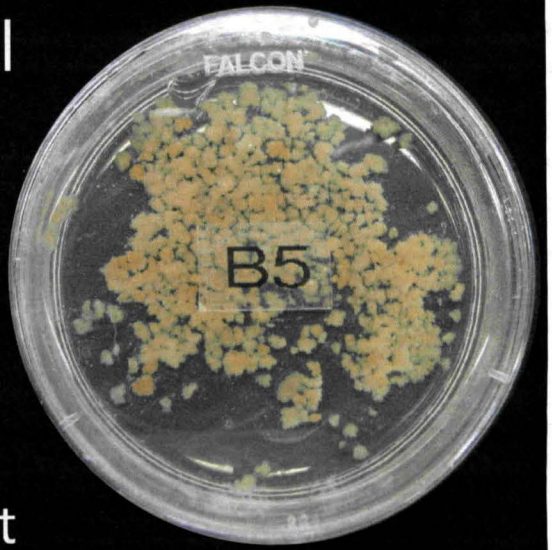
Hypothesis

We hypothesize that the unique response of cultured cells to spaceflight is caused by inappropriate modulation of typical gravity perception pathways.

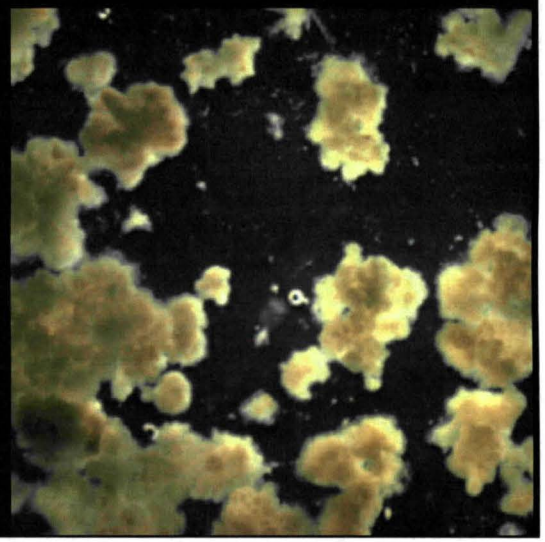
- Undifferentiated cells in BRIC-16 responded differently and anomalously to the spaceflight environment as compared to intact seedling arabidopsis plants.
  - The response was dramatic (5 – 10x greater levels of differential expression) compared to seedlings
  - The response was predominantly in heat shock / chaperone pathways typically not associated with gravity perception

Investigation Goals and Objectives

Our goal is to dissect the sensory and signal mechanism(s) that cell cultures use to detect and respond to spaceflight.



- What is it about undifferentiated cell cultures that makes them respond to the spaceflight environment in such a novel manner?
- Does the the lack of sensing organs and specialized cells disable a natural feedback system to check certain stress-response pathways?
- We will use molecular tools and mutant cell lines to investigate these questions.



Measurement approach (testing the Hypothesis)

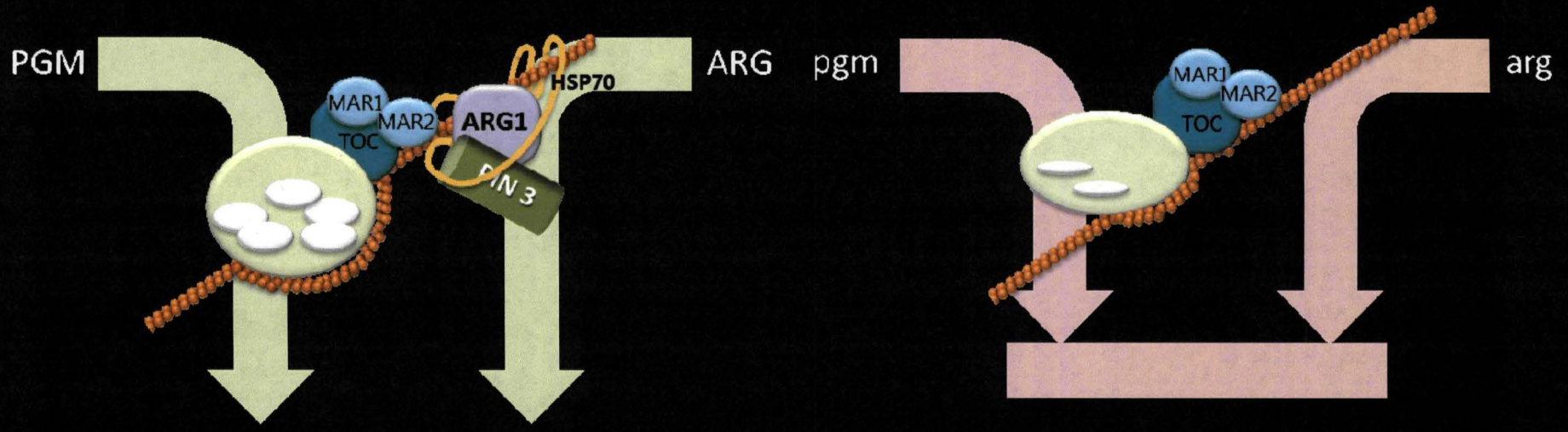
We will measure the adaptive changes imposed by spaceflight by evaluating by alterations in gene expression.

- We will do this by creating cultures from Arabidopsis plant lines with well characterized mutations in the gravity sensing and signaling genes: ARG1 and PGM1.
  - If cell cultures are using these typical sensing and signaling elements, the mutations should greatly attenuate the cell culture responses.
  - If cell cultures mount a response through some alternate pathway, these mutations should have no effect on the spaceflight response.
- The "Readout" will be in the patterns of gene expression as evaluated with genome-wide gene expression profiling (microarrays and RNAseq)

Plant lines and Molecular Approaches

Measurement approach

- A simplified model based on current understanding suggesting ways in which PGM and ARG contribute to gravity perception and signal transduction pathways to condition a typical gravity response in plants in 1g.
- We hypothesize that these same perception and signal pathways may be those that condition the spaceflight chaperone induction response. If so, mutations in those pathways should abolish the spaceflight chaperone induction response.



Wild-type

Mutants

Importance and reason for ISS

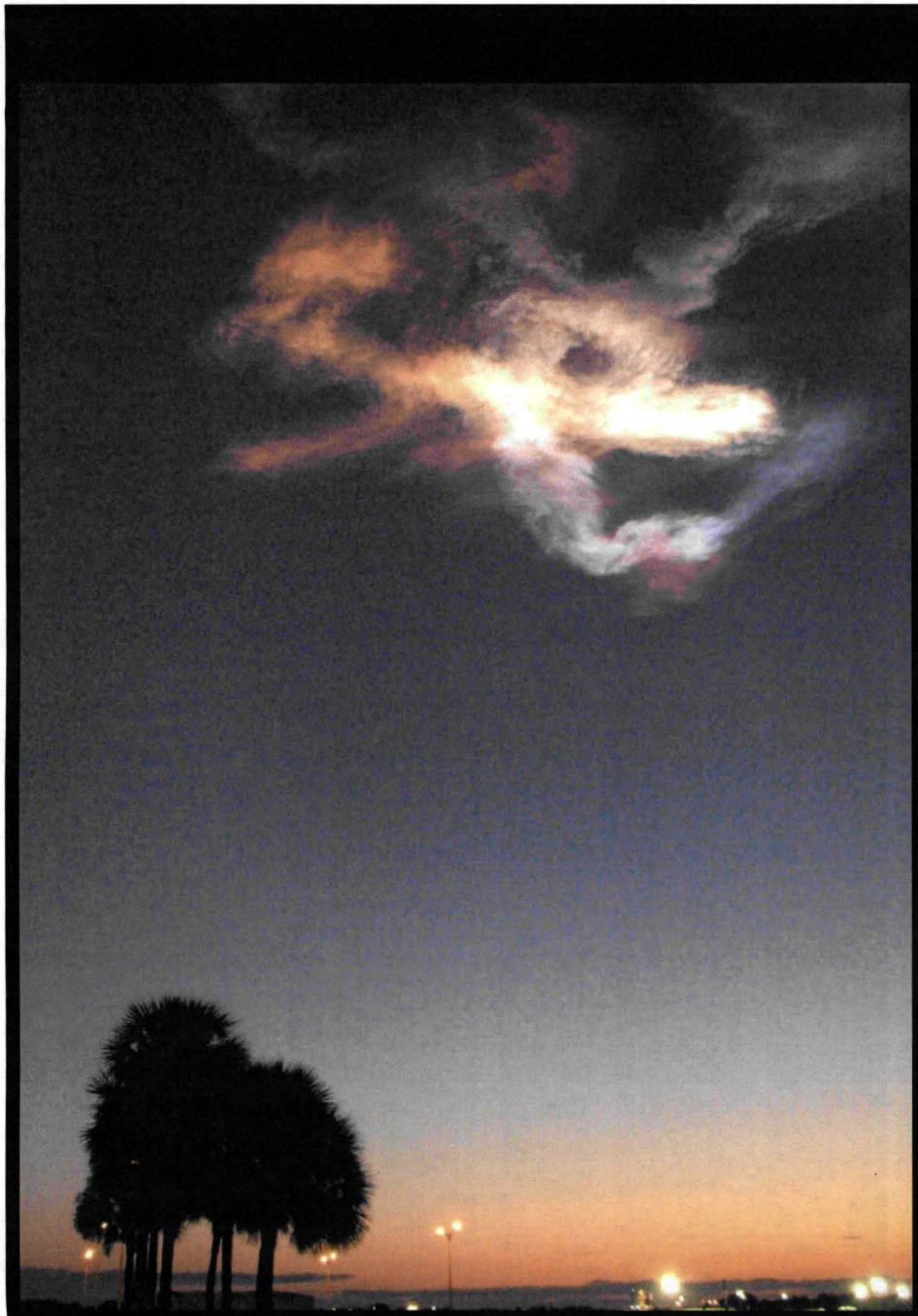
These experiments require a sustained microgravity environment, and embody the ISS science mission.

- Currently the only platform capable of supporting this experiment is the International Space Station.
- This experiment is hypothesis driven, and builds directly on results from a unique microgravity experiment; there is no other way to directly test the hypotheses provoked by the fist flight experiment.
- This experiment also represents the type of replicated science that was envisioned for the ISS during the development of the station.

Expected results – how they will advance the field and benefit Earth

The expected significance of this project is that it will advance the fundamental understanding of how plants perceive and process novel signals from the spaceflight environment.

- The undifferentiated cell cultures enables us to evaluate the fundamental mechanisms associated with plant responses to the novel environment of spaceflight.
- Help understand the mechanisms of environmental sensing at the molecular level – how important are the feed-back signals provided by differentiated sensing organs? What is the effect of removing the stimulus that fostered the evolution of those sensing organs?
- Insight into the underlying mechanisms of environmental sensing is an area recognized as fundamental within the Decadal Study.



- BRIC-16 publication thus far:

Paul, A.L., Zupanska, A.K., Ostrow, D.T., Zhang, Y., Sun, Y., Li, J.L., Shanker, S., Farmerie, W.G., Amalfitano, C.E., and Ferl, R.J. (2012). Spaceflight transcriptomes: unique responses to a novel environment. *Astrobiology* 12, 40-56.

- PIs' Contact Information:

Dr. Anna-Lisa Paul – PI
alp@ufl.edu
Department of Horticultural Sciences
Program in Plant Molecular and Cellular Biology
University of Florida
Gainesville, FL 32611-0690

Dr. Robert Ferl – CoPI
robferl@ufl.edu
Interdisciplinary Center for Biotechnology Research
Program in Plant Molecular and Cellular Biology
University of Florida
Gainesville, FL 32610