The NASA Specialized Center of Research and Training in Gravitational Biology at North Carolina State University.

Calcium, Signaling and Gravity: An Integrated Molecular, Cellular and Physiological Approach to Plant Gravitational Biology

Task Book Report for FY 2000

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Overview

The NSCORT is a consortium of 11 project leaders from 4 institutions including North Carolina State University (College of Agriculture and Life Sciences and School of Forestry), Wake Forest University, Baruch College (City University of New York), and Dynamac Corporation (which manages the Life Science support contract at NASA's Kennedy Space Center). Faculty, staff and students from all of these institutions participate in various aspects of the program. Participation by non-NASA supported personnel has added to the value of the grant. This report summarizes the progress of the NSCORT in FY2000. The overall mission of the NSCORT is spelled out in the two sub-headings: **Research** and **Education/Public Outreach.**

Research

The overall goals of the research are to advance the basic understanding of the role of calcium in gravity perception, induction and response and to alter gravity-induced growth by altering calcium homeostasis. The group uses an integrated molecular, cellular, and physiological approach to plant gravitational biology. The overall hypothesis is that calcium plays a major role in the

response of plants to gravity and if we alter the levels of calcium within a plant (genetically and pharmacalogically) the ability of the plant to respond to gravity will be altered.

In order to tackle the complex problem of the role of calcium homeostasis in plant gravitational biology, researchers came together over five projects. We have chosen two major systems for our studies: *Arabidopsis* for the molecular studies and the maize pulvinus for the biochemical studies. However, where it was necessary to solve a specific problem or where we have existing expertise, other plant systems were used. The research synergy is such that there are multiple co-investigators participating in each of the five projects. This approach has resulted in highly integrated research thrusts, listed below as Projects I - V:

Project I. Changing calcium homeostasis in transgenic plants: A reverse genetics approach (Robertson, Thompson, Boss)
Project II. Isolation of a novel class of mutants with impaired transduction of the gravity signal (Robertson, Muday);
Project III. Changes in gene expression during gravistimulation (Davies, Whetten, Sederoff);
Project IV. Signal transduction pathways and early responses to gravistimulation (Allen, Tucker, Boss, Muday, Davies, Whetten);
Project V. Effects of gravity on metabolism (Huber, Brown, Muday).

Project I of the NASA Specialized Center of Research and Training in Gravitational Biology at NC State University:

Changing calcium homeostasis in transgenic plants: A reverse genetics approach.

Dominique Robertson - NC State University
William Thompson - NC State University
Wendy Boss - NC State University

This research effort is one of five integrated projects that in their totality comprise the NSCORT in Gravitational Biology. Project I takes a reverse genetics approach to alter Ca^{2+} homeostasis in plants. The project has resulted in a number of constructs. These include calreticulin, calmodulin, calmodulin-binding peptides, PIP 5-kinases and the ER Ca^{2+}-ATPase under the control of inducible and targeted promoters. The work (research) has furnished the first evidence to support our hypothesis insofar as transgenic *Arabidopsis* plants over-expressing calreticulin in the endoplasmic reticulum, when grown on low calcium medium, have an enhanced response to gravity compared to controls (Wyatt et al., 2000; Persson et al., 2000). As part of this reverse genetics approach we produced the first stable transformants with a reliable, inducible Top10 promoter in *Arabidopsis* and optimized conditions for its use (Love et al., 2000). We also optimized conditions for using onion epidermal peels for transient expression (Scott et al., 1999). The onion system greatly facilitates imaging GFP-fusion proteins for localization studies and for testing our constructs. Another focus of the molecular group has been the optimization of calcium- and pH- sensitive reporter proteins for expression in *Arabidopsis* to facilitate analysis of ion fluxes in response to gravity.

Project II of the NASA Specialized Center of Research and Training in Gravitational Biology at NC State University:

Isolation of a novel class of mutants with impaired transduction of the gravity signal.

Dominique Robertson - NC State University
Gloria Muday - Wake Forest University

This research effort is one of five integrated projects that in their totality comprise the NSCORT in Gravitational Biology. Project II is a mutant screen of T-DNA tagged Arabidopsis that takes advantage of the fact that inflorescence stems can perceive but not respond to gravity at 4°C. We have recovered 8 mutants that have been screened through the F2 generation and we are in the process of characterizing these mutants. The mutants are proposed to have lesions in their **G**ravit**y P**ersistent **Si**gnaling mechanism and, therefore, they have been called GyPSi mutants (Wyatt et al., 2000). We anticipate that the mutant screen will lead to new insights concerning control points for gravi-sensing.

Project III of the NASA Specialized Center of Research and Training in Gravitational Biology at NC State University:

Changes in gene expression during gravistimulation.

Eric Davies - NC State University
Ross Whetten - NC State University
Ronald Sederoff - NC State University

This research effort is one of five integrated projects that in their totality comprise the NSCORT in Gravitational Biology. Project III describes another approach to identifying novel genes involved in gravity-induced signal transduction and response. Changes in transcriptio are indicated by the fact that specific transcripts become more abundant in gravi-stimulated pine xylem and maize pulvinus tissue, while changes in translation are indicated by differences in polysome profiles in gravi-stimulated maize pulvini. Our data suggest that transient changes in gene expression occur over time in the upper and lower halves of gravi-stimulated maize pulvini and are consistent with biochemical differences reported in Project IV and V. We also are using microarrays to identify genes expressed in gravi-stimulated pine and will eventually expand these studies to maize.

Project IV of the NASA Specialized Center of Research and Training in Gravitational Biology at NC State University:

Signal transduction pathways and early responses to gravistimulation.

Nina Allen - NC State University
Edward Tucker - Baruch College, City University of New York
Wendy Boss - NC State University
Gloria Muday - Wake Forest University
Eric Davies - NC State University
Ross Whetten - NC State University

This research effort is one of five integrated projects that in their totality comprise the NSCORT in Gravitational Biology. Project IV addresses cellular and biochemical studies of signal transduction pathways. We have evidence for a role for the phosphoinositide pathway and MAP kinase-regulated signaling events in gravi-stimulated maize pulvini. The rapid (10s) changes in inositol triophosphate (IP3) evident in the lower side of the maize pulvinus indicate an early

sensing of the gravity vector (Perera et al., 1999). In addition, we have repeatedly detected an oscillation of the metabolic changes alternating between the lower and upper sides of the pulvinus as though the commitment to elongate does not occur until after about 2-4h of gravi-stimulation. Although the maize pulvinus has been ideal for biochemical studies it is not tractable for imaging. To this end, we developed a method for isolating files of amyloplast-enriched bundle sheath cells that appear to be far better for imaging changes in cytosolic calcium and pH during gravi-stimulation. Although others were not able to detect changes in Ca^{2+} in gravi-stimulated root columella cells, we have shown a rapid (<1min) increase in pH of the Tier 2 columella cells on the lower side of Arabidopsis roots (Scott & Allen, 1999). To further study the role of auxin transport in root gravitropism, a system for separating and growing gravity-responsive root tips was developed (. In addition, proteins that bind and potentially regulate auxin transport proteins were found to bind F-actin affinity columns suggesting actin localization of the transport proteins is a potential means for regulating auxin transport (Butler et al., 1998 Muday, 2000). In order to measure calcium influx in response to gravity, we used a self-referencing Ca^{2+}-selective ion probe and monitored Ca^{2+}-influx into the tip cell of a filament of *Physcomitrella*. This cell has proven to be useful for microinjecting dyes and recombinant proteins, and studies of Ca^{2+} revealed a dramatic and rapid effect of light on cytosolic Ca^{2+} which may override gravity-induced changes.

Project V of the NASA Specialized Center of Research and Training in Gravitational Biology at NC State University:

Effects of gravity on metabolism.

Steven Huber - NC State University
Gloria Muday - Wake Forest University
Christopher Brown - Dynamac Corporation and NC State University

This research effort is one of five integrated projects that in their totality comprise the NSCORT in Gravitational Biology. Project V focuses on the role of carbon metabolism in the gravity response and studies of starch metabolism in space flight. Rapid (10 min) changes in malate concentrations are evident in the maize pulvinus following gravi-stimulation. Changes in the intracellular distribution of the sucrose metabolizing enzyme, sucrose synthase, were also noted suggesting a mechanism regulated by protein phosphorylation that affects the membrane and cytoskeletal localization of the enzyme (Winter et al., 1998 a; b; Winter & Huber, 2000). The effect of gravity on the regulation of invertase activity as well as transcription of its gene has become a major focus of this work (Long et al., 2000). The potential for changes in cytosolic pH to regulate invertase activity and a possible connection to the cytoskeletal structure make this a very exciting system for regulating a growth response.

Plants grown in space have been studied with regard to their starch metabolism and were found to produce less starch and have smaller starch granules (Klymchuk, et al.,1999; Kuznetsov et al., 2000). The comparative studies indicate that these effects resulted from space flight itself and not from the production of the plant hormone, ethylene, which is often high in the space shuttle.

Education/Public Outreach

Christopher S. Brown - Dynamac Corporation and NC State University

In addition to its research goals, the NCSU NSCORT has established a vibrant E/PO program that has provided opportunities for educators, students and the general public to enhance their appreciation of plant space biology in particular and science in general. Through visits to schools for demonstrations/lectures, a special topics course for high school teachers, a college level

course in Space Biology and participation in community science activities, the NCSU NSCORT has interacted with over 3,000 students, 600 teachers and 500 members of the general public during the last four years. In order to expand and prolong the ability to reach out, especially to educators, we have developed a web-based resource containing information and laboratory exercises relating to plants, gravity and space (www.cals.ncsu.edu/pgs) and a website for the delivery of material for a college course in Space Biology (www.cals.ncsu.edu/course/bo277).