

**The NSCORT at NC State University
Calcium, Signaling and Gravity: An Integrated Molecular, Cellular and Physiological
Approach to Plant Gravitational Biology**



**NASA Office of Biological and Physical Research Task Book Report 2002
Progress from October 1, 2001 – September 30, 2002**

BACKGROUND / HISTORY

The NASA Specialized Center of Research and Training (NSCORT) in Gravitational Biology was established at N.C. State University in 1996. The multi-year grant was awarded to N.C. State University, Wake Forest University, the Dynamac Corporation and Baruch College (City University of NY) in a competitive process (NRA 94-OLMSA-04). This group centers its efforts on research and training involving "Calcium, Signaling and Gravity: An Integrated Molecular, Cellular and Physiological Approach to Plant Gravitational Biology".

Since its inception, the NSCORT has supported research for 11 Project Leaders, 20 postdoctoral and graduate students and dozens of undergraduate students at three universities and one private company resulting in over 175 research publications/published abstracts. Additionally, the NSCORT developed a vibrant education and outreach program that has reached nearly 6,000 teachers, students and members of the general public. The NC State University NSCORT has become internationally recognized for its contributions to plant gravitational and space biology research bringing wide respect to NASA and to the discipline of gravitational biology through articles in peer reviewed journals, invited lectures and reports by leading media organizations.

ABSTRACT

The NSCORT in Plant Gravitational Biology 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. Faculty, staff and students from all of these institutions participate in various aspects of the program. In addition, participation by non-NASA supported personnel has added to the value of the grant. This report summarizes the progress of the NSCORT from October 1, 2001 through September 30, 2002. The overall mission of the NSCORT is spelled out under the two sub-headings: Research and Education/Outreach.

Research:

The overarching goal of the research at the NSCORT in Plant Gravitational Biology is to advance the basic understanding of the events involved in plant gravity sensing and response. The approach we have taken is to investigate the role of calcium in gravity sensing, induction and response and to alter gravity-induced growth by altering calcium homeostasis. The group uses an integrated molecular, cellular, and physiological approach in attacking these fundamental studies. During this year, the NSCORT group published 19 articles in peer-reviewed scientific journals.

Education/Outreach:

Education and public outreach is an integral facet of the NSCORT in Plant Gravitational Biology. An innovative learning environment has been created for postdoctoral fellows, graduate and undergraduate students through active integration of teaching, research and outreach opportunities. Enthusiastic appreciation for gravitational plant biology has been communicated to thousands of individuals, including peers in the scientific community, university and K-12 students, teachers, and members of the general public. During this year, the NSCORT group interacted with over 900 students and 60 teachers in formal and informal settings.

PROGRESS FOR THE CURRENT YEAR

Research

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 (Thompson, Robertson and Boss).

This research effort is one of five integrated projects that in their totality comprise the NSCORT in Gravitational Biology. As part of the reverse genetic approach to study the role of phosphoinositide signaling in plant gravitropic responses, we are producing *Arabidopsis* plants transformed with genes encoding either the *Arabidopsis* phosphatidylinositol phosphate 5-kinase or the human type I inositol polyphosphate 5'phosphatase. The gravitropic responses of T4 generation plants are currently under investigation. The inositol polyphosphate 5'phosphatase was also expressed in tobacco cell cultures, where changes in phosphoinositide metabolism were characterized biochemically (Perera et al., 2002).

To investigate the effect of Ca^{++} storage and localization by over-expressed calreticulin calcium-binding peptides (CBP), we have now produced transgenic *Arabidopsis* lines expressing these peptides as ER-targeted GFP fusions (ER-CBP). Interestingly, we found that constitutive expression of a GFP fusion with the ER-CBP can increase calcium stores resulting in higher tissue levels of calcium (Wyatt, et al., 2001, Tsou, 2001). Similar constructs tested in primary maize transformants demonstrated that increased levels of calcium accumulated in transgenic ER-CBP plants compared to GFP control transgenics. In this trial, plants were grown under standard conditions in soil. We are now analyzing the gravity response in the *Arabidopsis* transformants that constitutively overproduce the C-terminal domain of calreticulin, the calcium binding peptide (CBP). As these plants carry the CBP as a fusion protein with green fluorescent protein (GFP), all analyses of the transgenics have used this reporter to verify expression of the transgene. These plants have a two-fold reduction in root gravitropism when grown on media with no added calcium and this inhibition is not observed when 10 mM calcium is added back to the media, suggesting that the free levels of Ca^{++} are important in the intensity of the gravitropic response. Additional experiments are examining auxin transport in these transgenics under low, high, or low returned to high calcium conditions. A reproducible delay in the gravitropic response of about 30 mins was seen in roots of *Arabidopsis* carrying the CBP-GFP fusion construct compared to GFP controls. As might be predicted for plants with high calcium stores, these same plants show better growth than GFP controls after transfer to high sodium chloride (0.1 - 0.2 M salt) (Tsou, 2002, Patent Application No. PCT/US01/13563).

A third project was stimulated by our need for high-resolution analysis of the free Ca^{++} distribution in living cells. In this project, we have produced greatly improved 'cameleon' genes that function as sensitive *in vivo* reporters of free Ca^{++} concentration (Love et al. in preparation). In these chimeric genes, different variants of the GFP fluorophore are linked to opposite ends of a calmodulin molecule. Free Ca^{++} influences the conformation of the calmodulin, leading to changes in fluorescence resonance energy transfer (FRET) between the two fluorophores. FRET patterns can then be used to obtain a map of Ca concentration in living cells. The new cameleon construct permits high resolution, real time imaging of Ca^{++} concentrations at the subcellular, cellular, or tissue level, using confocal microscopy or high resolution multiphoton imaging techniques.

Project II. Isolation of a novel class of Arabidopsis mutants with impaired transduction of the gravity signal (Robertson and Muday).

This research effort is one of five integrated projects that in their totality comprise the NSCORT in Gravitational Biology. A mutant screen of T-DNA tagged *Arabidopsis* was performed that took advantage of the fact that inflorescence stems can perceive but not respond to gravity at 4°C. We have recovered 8 mutants, each of which show a normal response to gravity at room temperature, and have done genetic tests to segregate them into three different groups. The mutants are proposed to have lesions in their Gravity Persistent Signaling mechanism and, therefore, they have been called GPS mutants. The three mutant phenotypes are; a reverse response to gravity; a hyper-response to gravity; and a lack of response to gravity. Two of these are completely novel phenotypes that can only be explained as being defective in sensing or signal transduction (Wyatt et al. 2002, in press). The mutant genes are being cloned by Dr. Sarah Wyatt of Ohio University, formerly a postdoctoral fellow with the NSCORT. Additionally, characterization of these mutants continues as collaboration between the Wyatt and Muday laboratories. The cold treatment prevents polar auxin transport as well as gravity response, but does not impair perception of gravity in wild-type. Study of the effects of cold treatments and gravity stimulation on polar auxin transport in the wild-type and *gps1,2* and 3 inflorescences is currently underway.

Project III. Changes in plant gene expression during and after gravistimulation (Davies, Sederoff, Whetten).

This research effort is one of five integrated projects that in their totality comprise the NSCORT in Gravitational Biology. As such, Project III is focused on identifying those genes whose expression is altered during and after gravity-stimulation of pine (compression wood) and maize plants (pulvinus). We are identifying genes whose expression changes (either up-regulated or down-regulated) and which might be involved in the sensing stage, the signal transduction phase and/or the growth response. We are also looking at changes occurring in the very early stages of sensing/transduction at the level of transcription as well as translation and in genes that are expressed unequally on the upper and lower sides of gravistimulated plants.

In the maize pulvinus, we have identified two transcripts encoding calcium-responsive proteins whose level increases in both sides, but whose recruitment into polysomes is greater and faster (within 15 minutes) on the lower side (Heilmann et al. 2001). This is the most rapid translational response reported after gravi-stimulation in the maize pulvinus system to date and may be one of the fastest in any plant system challenged by an altered gravity stimulus. We have also shown that the asymmetric increases in polysomes (more on lower than upper side) occur not only in the growth-responsive pulvinus, but also in the non-responsive pulvinus. Apparently the non-responsive pulvinus can sense gravity even though it does not exhibit a growth response. We are now looking to see if the same transcripts are made and recruited into polysomes in both kinds of pulvini.

With pine, we have looked primarily at transcriptional changes and are using microarray analysis to identify the totality and complexity of global changes (Sun 2001; Whetten et al. 2001). We have also focused on the genes involved in the growth response, especially those related to lignin biosynthesis, since pine is the model system for such studies and compression wood is a major practical problem in the pulp and paper industry. The use of microarray technology is now being expanded to include maize and Arabidopsis.

Project IV. Signal transduction pathways and early responses to gravistimulation in plants (Boss, Allen, Muday, Davies, Muday Whetten and Tucker).

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 in gravity stimulated plants. We have used three model systems to study the signaling pathways: 1) maize pulvini; 2) Arabidopsis roots; and 3) *Physcomytrella patens* caulonema. The group has published the following new insights within the past year:

1) Maize and oat pulvini:

A) *Rapid changes in pH are evident only in the amyloplast-containing bundle sheath cells of the maize pulvinus and not in the parenchyma cells.* There was a decrease in the pH of the cytoplasm near the sides of the cells gravi-stimulated pulvinus where changes in amyloplast orientation were observed. In contrast, the pH of the cytoplasm at the base of the cell where the amyloplast accumulated became more basic (Johannes et al. 2001).

B) *Rapid changes occur in selective mRNA recruited to polysomes isolated from the upper and lower sides of maize pulvini within minutes of gravi-stimulation* (described in Project III; Heilmann et al., 2001).

C) *A differential increase in auxin occurs on the lower side of the pulvinus after the commitment to differential growth, but prior to growth and the increase in invertase* (Long et al., 2002).

D) *The actin cytoskeleton is present in root columella cells putting to rest the controversy as to whether the gravi-sensing cells of the root contain a functional cytoskeletal network.* (Collings et al., 2001).

2) Arabidopsis:

A) *Reversible protein phosphorylation can regulate auxin transport.* A genetic and pharmacological approach was used to show that if protein phosphatase 2A activity was reduced, basipetal auxin transport was increased and there were defects in differential cell elongation. Reducing basipetal auxin transport with the inhibitor, NPA, restored the normal gravitropic response. Studies of gravitropism and lateral root growth indicated differential regulation of acropetal and basipetal auxin transport (Rashotte et al 2000 and 2001; reviewed in Muday and DeLong, 2001; Muday 2001).

B) *Flavonoids can function as negative regulators of auxin transport in vivo.* Mutants with reduced flavonoid biosynthesis had increased auxin transport and the additional of flavonoids slowed down auxin transport. (Peer et al., 2001; Brown et al., 2000).

C) *Comparison of IAA and IBA transport.* The transport of the two endogenous auxins, indole 3-acetic acid (IAA) and indole 3-butyric acid (IBA) were compared in several tissues of Arabidopsis in order to understand the similarities and differences in the movement of these two auxins. Both auxins are important in gravitropic bending, as mutations that reduce the response to either auxin impair root gravitropic bending. IBA moves in roots in similar directions and with similar rates, but uses different transporters that are not sensitive to inhibition by IAA efflux inhibitors (Rashotte et al. 2002)

D) *Examination of gravity response in calreticulin transgenics.* We are currently analyzing the gravity response in plants that constitutively overproduce the calcium binding domain (C-domain) of calreticulin. These plants have a defect in root gravitropism when grown under limiting calcium and this defect is not observed under conditions where calcium is abundant.

E.) *A genetic approach was used to alter InsP₃ levels in plants.* To test the hypothesis that a differential long term increase in InsP₃ was necessary for gravitropism, cells and plants were transformed with the human gene encoding InsP 5-ptase which when expressed should metabolize InsP₃ rapidly. Biochemical characterization of the transformed cells grown in culture has been published (Perera et al., 2002). We are currently characterizing the transgenic Arabidopsis plants.

3) Physcomytrella patens:

A) *UV-A light induces calcium waves in Physcomytrella patens.* *In vivo* analysis of cytosolic calcium indicated that blue/UV-A light used for the ratiometric measurements induced calcium waves and thus precluded measurements of changes in calcium in response to gravity (Tucker, submitted).

B) *Cryptochrome mutants of Physcomytrella patens can be used to distinguish the role of calcium in blue and UV-A light responses.* Light-insensitive mutants have been obtained and are being used to study the role of calcium in gravi-sensing (Tucker et al., in preparation).

C) *Cytokinins affect the direction of the gravitropic response as well as branching.* Knockout mutants with selective deficiencies in auxin and cytokinin biosynthesis as well as calcium-binding proteins are being selected to delineate these aspects of the gravitropic response (Tucker et al., in preparation).

Project V. Effects of gravity on plant metabolism (Huber, Muday and Brown).

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 two model systems have been studied within the past year.

1). Enzymes of sucrose metabolism:

Previously, changes in the intracellular distribution of the sucrose metabolizing enzyme, sucrose synthase, were noted suggesting a mechanism regulated by protein phosphorylation that affects the membrane and cytoskeletal localization of the enzyme (Winter and Huber 2000). Studies have focused on sucrose synthase, which is an important enzyme of sucrose metabolism in growing plant tissues such as the maize leaf elongation zone and the graviresponding maize pulvinus. Sequence analysis of the enzyme suggested that serine-170 may be a phosphorylation site, along with the previously identified major phosphorylation site at serine-15. To test this, antibodies were produced that specifically recognized the sucrose synthase protein when phosphorylated at the serine-170 site. The results indicate that this site can be readily phosphorylated, but in vivo, the site is only weakly phosphorylated. It appears that phosphorylation in vivo may be restricted by other proteins that bind to sucrose synthase. Further, when the serine-170 site is phosphorylated, sucrose synthase is "marked" for degradation by the proteasome system. This could be one mechanism that regulates the level of the sucrose synthase protein in various plant tissues (Hardin et al. in press)

2). Starch granule production:

The effect of spaceflight on starch development in soybean cotyledons and potato tubers was compared to ground controls. In general, starch grains from both species were 20-50% smaller than the ground controls. The densities were similar, but the magnetic susceptibility (movement of starch grains in a magnetic field) was altered, indicating changes in the starch structure. There was increased amylose content and an increase in the rate of starch degradation (from isolated grains) in the space tissue compared to the ground controls. Higher levels of ethylene were measured post-flight in the space exposed plants, suggesting that ethylene is playing a role (Kuznetsov et al. 2001)

Education and Public Outreach (Brown and Thornton)

Between October 1, 2001 and September 30, 2002, NSCORT scientists engaged over 900 students and over 60 teachers and faculty members in thinking about and learning concepts related to plant biology and space. This year's outreach efforts brings the totals since the inception of the NSCORT in 1996 to over 4600 students, 700 teachers, and 500 members of the general public. Several noteworthy accomplishments and activities have taken place in the NSCORT education and public outreach efforts this year.

Since February 2002, NSCORT has been supporting 20% of the time for an Educational Program Coordinator, Courtney Thornton, in collaboration with the Kenan Institute for Engineering, Technology and Science at NC State University. Courtney focuses on coordinating all outreach events and proactively seeking new opportunities to share NSCORT information with the public.

The enrollment in Space Biology, an undergraduate course offered at NC State University had its highest enrollment ever in the Fall of 2002 with 32 students as well as participation by 7 guest lecturers (www.cals.ncsu.edu/course/bo277).

The NSCORT has traditionally provided outreach to the K-12 and undergraduate student communities. Another segment of the general public interested in advances in space research is the senior adult population. In order to actively address this population, a relationship has been forged with Encore, a lifelong learning initiative of NC State University for adults over 50 years of age. NSCORT Director Chris Brown and collaborators from NC Space Grant Consortium are preparing to present a six-week lecture series entitled "Space Exploration: From Astronomy to

Zoology" in January and February of 2003. With help from Encore, additional contacts have been made to local retirement communities with educationally focused programming.

Fully 45% of the total number of students reached in this fiscal year were from North Carolina's third grade classrooms. This significant statistic results from the fact that the third grade science curriculum includes four competencies, all of which are related to NSCORT: plant adaptations, soil inquiry, heat and light, and the sun/moon/earth system. In August 2002, NSCORT surveyed Triangle area third grade teachers to determine their needs in teaching the science curriculum and to gauge their interest in a yearlong, interdisciplinary curriculum based on a theme of supporting life in space. Interest was high, and a focus group met with the Educational Program Coordinator in late September to begin work on this concept.

Throughout the year, the NSCORT website is maintained and kept current. In addition this year, all NSCORT-sponsored publications and media releases were collected and organized in paper form. This information is currently being transferred to digital format and onto CDs with the goal of being able to distribute NSCORT publications and press to parties interested in learning more about NSCORT and the research generated by its members.

RESEARCH IMPACT ON AMERICA / EARTH BENEFITS

This research will determine the mechanisms by which plants sense and respond to several environmental stimuli, especially gravity. It will provide a fundamental understanding of basic plant processes, especially at the cellular, molecular, and developmental levels. A deeper understanding of how plants respond to gravity and other environmental conditions will improve our understanding of how they grow in various space conditions (e.g. Earth orbit, Mars) and how their growth can be modified to maximize yields on Earth. More applied work on specific plants should yield valuable by-products such as the ability of plants to grow on agriculturally marginal soils (transgenic plants with increased calcium stores, Patent no. 09/844,066), enhanced paper quality (e.g., pine seedling system and its formation of compression wood), yield of seed grains (e.g., reorientation of corn plants blown over in strong winds), improved quality of cut flowers (maintenance of straight stems during long distance shipping), and nutraceutical plants (e.g., plants with enhanced capability of storing essential nutrients such as calcium).

As members of NSCORT team, we are committed to a comprehensive approach to the enhancement of science education at all levels and the public understanding of science. We actively promote and participate in programs to further the Education and Public Outreach (E/PO) goals of NASA and the HEDS/OBPR Enterprises. Through visits to schools for demonstrations/lectures, a special topics course for high school teachers, a college level course in Space Biology, participation in community science activities and interactions with various press outlets, the NSCORT in Plant Gravitational Biology has interacted with thousands of students, teachers and members of the general public since its inception in 1996.

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This report compiled on October 31, 2002 by Chris Brown with input from all the members of the NSCORT team.