

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

**NSCORT Task Book 2001
Progress from October 1, 2000 – September 30, 2001**

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 centered its efforts on research and training involving "Calcium, Signaling and Gravity: An Integrated Molecular, Cellular and Physiological Approach to Plant Gravitational Biology".

This highly successful has supported research for 11 Project Leaders, 20 postdoctoral and graduate students and dozens of undergraduate students at three universities and one private company and has resulted in over 170 research publications/published abstracts. Additionally, the NSCORT developed a vibrant education and outreach program that has reached nearly 5,000 teachers, students and 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, 2000 through September 30, 2001. 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.

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.

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. T2 generation plants are currently being screened for gene expression, and T3 generation plants will be used to study gravitropic responses. The inositol polyphosphate 5'phosphatase was also expressed in tobacco cell cultures, where changes in phosphoinositide metabolism were characterized biochemically. To investigate the effect of Ca⁺⁺ binding 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, our initial results suggest that ER-CBP can augment plant stress responses to high salt conditions (Wyatt et al. 2001; Tsou et al., in preparation). To investigate the effect of altered Ca⁺⁺ stores on gravitropism, ER-CBP plants are being crossed with *Arabidopsis* lines containing modified calcium signaling proteins. Similar constructs are being also tested in maize where changes in gravitropic response can be more effectively separated from changes in growth. 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. 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 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 GyPSi 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. 2001 submitted). We are now cloning these mutant genes in collaboration with Dr Sarah Wyatt of Ohio University, formerly a postdoctoral fellow with the NSCORT.

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. 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. Use of microarray technology is now being expanded to include maize.

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

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 and oat pulvini; 2) Arabidopsis roots; and 3) *Physcomyrella 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., Plant Physiology).

B) *A differential increase in $InsP_3$ appears to be necessary for differential growth.* Upon gravi-stimulation, rapid and long-term changes in $InsP_3$ were observed in gravi-responsive oat pulvini but not in the non-gravi-responsive internodal tissue. The long-term differential increase in $InsP_3$ in the pulvini and gravitropic bending are inhibited by a pharmacological inhibitor of phospholipase C (Perera et al., 2001).

C) *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).

D) *MAP kinase activity changes in the upper and lower side of the gravi-stimulated maize pulvinus ultimately showing a steady increase on the lower side as the tissue makes a commitment to differential growth* (Clore et al. in preparation). SEE ROSS COMMENTS

E) *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* (described in Project V; Long et al., 2001).

F) *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 2000 and 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).

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. 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 and 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, Long et. al. 2001). Evidence obtained indicates that auxin redistribution, as a result of polar auxin transport, is necessary for gravity-stimulated transcription of the *Ivr2* gene (which encodes a soluble acid invertase) and differential cell elongation across the pulvinus that results in upward bending of the shoot. 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.

Education and Public Outreach (Brown)

In addition to its research accomplishments, 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 3,700 students, 650 teachers and 500 members of the general public during the last five 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).

The NSCORT in Plant Gravitational Biology hosted a symposium at the American Association for the Advancement of Science annual meeting in February, 2001. Drs. Nina Strömngren Allen and Chris Brown organized "Biology Into Space: A Matter of Some Gravity". The speakers at this symposium discussed the progress made to date in gravitational and space biology and the implications for future directions in research. This session was attended by national and

international members of the scientific community and press and resulted in numerous popular press pieces. The speakers at the symposium included:

- Dr. Don Ingber, Harvard Medical School - "The Cellular Basis of Mechanotransduction"
Dr. Patrick Masson, University of Wisconsin - "Molecular Mechanisms of Plant Responses to Gravity"
Dr. Wendy Boss, North Carolina State University - "Signal Transduction Pathways and Early Responses of Plants to Gravistimulation"
Dr. Robert Ferl, University of Florida - "Taking Plants into Space: Answering Fundamental Questions of Plant Stress"
Dr. Raymond Wheeler, NASA Kennedy Space Center - "Human Life Support for Space Missions Using a Bioregenerative System"

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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Patents

Patent Serial No. 09/844,006 Transgenic plants with increased calcium stores. Sarah E. Wyatt, Pei-Lan Tsou, Wendy F. Boss, and Dominique Robertson