

# Microgravity Research in Plant Biological Systems: Realizing the Potential of Molecular Biology

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## ABSTRACT

The sole all-pervasive feature of the environment that has helped shape, through evolution, all life on Earth is gravity. The near weightlessness of the Space Station Freedom space environment allows gravitational effects to be essentially uncoupled, thus providing an unprecedented opportunity to manipulate, systematically dissect, study, and exploit the role of gravity in the growth and development of all life forms. New and exciting opportunities are now available to utilize molecular biological and biochemical approaches to study the effects of microgravity on living organisms. By careful experimentation, we can determine how gravity perception occurs, how the resulting signals are produced and transduced, and how or if tissue-specific differences in gene expression occur. Microgravity research can provide unique new approaches to further our basic understanding of development and metabolic processes of cells and organisms, and to further the application of this new knowledge for the betterment of humankind.

## INTRODUCTION

Space Station Freedom (SSF) symbolizes a renaissance of NASA's goal to address fundamental questions pertaining to the effect of gravity on living organisms. Many gravitational effects, at least in a phenomenological sense, are already known or suspected, and hence should be amenable to scientific inquiry; others undoubtedly await discovery. SSF will be in service for more than 25 years, and will provide sustained access to a stable microgravity environment, which cannot be duplicated on Earth. Life science experiments on SSF will permit a systematic dissection and analysis, at the molecular and biochemical levels, of various biological phenomena (primarily developmental) that are apparently perturbed in the microgravity environment. Such studies will employ both cell and whole organisms, using all forms of life. It is anticipated that the investigations on SSF will not only benefit space biology, but will also provide novel fundamental and

needed knowledge for application to a broad spectrum of human needs. Thus, there exists an unusual opportunity to assess the effects of microgravity and other effects unique to spaceflight on biological processes — in particular, on plants, which display several pronounced gravitropic responses during their life cycles under normal gravitational conditions.

Plant life forms respond to gravitational influences (at 1 g) as demonstrated by gravitropic phenomena. For example, leaning herbaceous plants regain an upright position by increased longitudinal growth on the underside of the stem. Woody gymnosperm and angiosperm plants, on the other hand, restore vertical alignment via altered stem growth patterns resulting in the formation of compression (Timell, 1986; Fengel and Wegener, 1984) and tension (Fengel and Wegener, 1984) wood tissues, respectively, i.e., so-called reaction wood. Gravitropic effects displayed by roots are apparently correlated with the displacement of statoliths in the root tips (Krikorian and Levine, 1991; Volkman et al., 1991). Many examples of gravitational effects in plants have been described, and some of these phenomena have already been preliminarily studied in space (Halstead and Dutcher, 1987; Halstead et al., 1991). Microgravity experiments with plants ranging from unicellular algae to angiosperms have revealed differences in growth and development when compared with 1 g controls, particularly at the subcellular, cellular, and tissue/organ levels (Halstead and Dutcher, 1987; Halstead et al., 1991). These studies have revealed various phenomenological observations, including: alterations in endoplasmic reticula and ribosomes, "swollen" mitochondria, changes in morphology of the cisternae of dictyosomes, random distribution of amyloplasts (with smaller starch grains), multiple nuclei, chromosomal aberrations, reduction or (partial) inhibition of cell mitosis, disturbances in the mitotic spindle mechanism, differences in cell size and shape, diminution of cellular aggregation capability, alteration in rate(s) of differentiation presumed to lead to more rapid aging, thinner cells walls (with

apparently altered biopolymer composition and architecture), disoriented roots (growing upwards rather than downwards), and substantial differences in essential element composition. The inescapable conclusion is that microgravity has a profound effect on plant growth and development.

Phenomenal advances in several areas of plant science have occurred within the last two decades. Knowledge of the molecular biology, biochemistry, physiology, and cell biology of plants has entered a new era in which gene transfer technology has contributed to both fundamental knowledge of plants and to the application of this knowledge to agriculture and related industries. The incorporation of SSF opportunities into this new plant biology can provide an added dimension for interdisciplinary research on plants to answer fundamentally important questions heretofore not possible to address. The following discussions suggest some selected topics in which microgravity research might be the focus of interdisciplinary efforts to contribute new knowledge in areas of plant biology where gravity has been recognized to play major roles in plant growth and development.

### GRAVITY SENSING BY PLANTS

A variety of studies on geotropism strongly suggests that when the normal gravitational vector is displaced, a significant alteration of biochemical events occurs. Striking evidence for this assertion comes from biochemical (Lewis et al.) and chemical (Timell, 1986) analyses of reaction wood tissue cells in angiosperms and gymnosperms, which differ substantially in their biopolymer composition and cell wall assembly mechanisms/architecture when compared with normally growing counterparts. This indicates that there is a distinct gravity-sensing mechanism that is initiated perhaps by a perturbation in (mechanical) stress-fields experienced by the cytoskeleton. There are two possibilities for signal transduction: in one scenario, a signal molecule (or molecules) is (are) generated, and bind to one (or various) specific receptor site(s). In the other case, the changes in the stress-field affect conformational changes to the receptor molecule(s), thereby facilitating "docking" of the messenger molecule(s). In both situations, various biochemical events are amplified or repressed either directly or via modulation of gene expression (i.e., via inducing coordinate expression of multiple genes) to redirect a cascade of biochemical events. Although the entire area of gene/biochemical activation in response to the gravitational stimulus is virtually devoid of knowledge, this is an area that can be readily investigated through SSF activities.

### SIGNALLING

The signalling mechanisms that regulate genes involved in plant growth and development, plant defense, and host-parasite interactions are under intensive investigation worldwide. In terms of microgravity research, how the gravitational stimulus is transduced to affect biological processes is of fundamental importance to our understanding and exploitation of plant growth and development. It is noteworthy that when this stimulus is essentially removed, a perturbation of normal growth and development follows. This has been elegantly shown by experiments with pine, oats, and mung bean seedlings in the Space Shuttle, where it was observed that the roots were disoriented in microgravity (Cowles et al., 1989). Many researchers have attempted to explain gravitropism in terms of a free-falling statolith in the cytosol coming to rest on the cytoskeleton surface. But how these collisions are subsequently transduced into modulation of gene expression/biochemical events (and resulting physiological responses) is unknown. It is possible that the statolith interaction with the cytoskeleton results in localized stress gradients or concentrations, as suggested above, and that either a chemical message is released (similar to the polypeptide hormone, systemin, released on insect attack (Pearce et al., 1991)), thereby activating a coordinated gene expression response, or the gradient affects a macromolecular conformational change, thereby facilitating binding of the signal molecule(s) to the receptor(s). Whatever scenario holds, a cascade of distinct, overlapping signalling events follows. Thus, incorporation of a coordinated research program involving SSF, utilizing known methodologies and concepts to study signal transduction leading to gravity-stimulation modulation by gene expression, could provide a novel, fundamental approach to furthering our knowledge of signal transduction in plants.

### IDENTIFYING GENES INVOLVED WITH GRAVITY COMPENSATION PROCESSES

Not even the simplest molecular biological experiments have been carried out in outer space to date. This is (primarily) because of our inability to cryogenically store plant tissue ( $-70^{\circ}\text{C}$ ) in orbit on spacecraft, or to isolate and store and manipulate labile compounds under such conditions. Yet, the descriptive changes reported in preliminary space experiments encompassing various aspects of altered growth and development are so striking that they demand our attention. Given the fact that cryogenic facilities will be placed on SSF, experiments using molecular biological techniques involving plant tissues and organs

can now be given a high priority, and changes in gene expression (induction or repression) and/or the causal and ensuing biochemical consequences that are influenced by microgravity can be investigated and determined. The next greatest research challenge and opportunity in space will be to manipulate genes in space, to establish how they are regulated, and to investigate their biochemical consequences.

### CELL-CELL RECOGNITION AND ADHESION

There is considerable and growing interest in how single cells eventually differentiate into different organs, first via recognition/adhesion interactions leading to pattern formation and, ultimately, via morphogenesis/differentiation (Siu, 1990; Wilkin and Curtis, 1990). That gravitational effects seem to play an important role in such processes has been concluded from several studies, e.g., *Daucus carota* protoplasts were observed to aggregate poorly in microgravity when compared with their 1 g counterparts (Rasmussen et al., 1990). Gravitational effects on cell-cell adhesion may be a more general phenomenon since poor aggregation in microgravity has also been observed with lymphocytes and red blood cells (Halstead et al., 1991). In plants (van Engelen et al., 1991) and animals, specific cell-recognition molecules are associated on the surface of individual cells targeted for aggregation (pattern formation). Given that this process is adversely affected in microgravity, the regulation and composition of cell surface components become logical targets for microgravity research. Current methodologies seem to be well suited for incorporation into an interdisciplinary project in this area, and should provide important information regarding cell patterning.

### CELL WALL SYNTHESIS

Plants have, as their major constituents, the cell wall components, i.e., cellulose, lignins, and hemicelluloses. During normal (1 g) growth and development, the plant produces various cell types with distinctive cell walls that differ in the composition and organization of their macromolecular substituents. (It is this process that distinguishes plant and animal cells.) But the biochemistry, including synthesis, deposition, and degradation processes, of these biopolymers is not fully understood, e.g., it is still unknown how cellulose, nature's most abundant organic polymer, is enzymatically synthesized, or how chain (microfibril) orientation is controlled and altered during cell wall synthesis. In a related matter, we do not understand how coordinate synthesis of lignin and hemicelluloses is regulated during cell

wall assembly (Lewis and Yamamoto, 1990). Equally lacking is an understanding of how primary wall assembly and expansion occur, or even how different cell (wall) tissue types are induced or controlled.

Experiments in space to this point (albeit preliminary) have suggested that biopolymer composition and their organization in the cell walls (i.e., architecture) is substantially perturbed in microgravity (Halstead and Dutcher, 1987; Halstead et al., 1991; Lewis et al.). Since the microgravity environment is free of the gravitational stimulus, it can be postulated that these cell walls represent the simplest architecture possible in the growing/developing plant. Thus, a determination of the factors controlling cell wall formation in microgravity will result in development of new strategies to biotechnologically manipulate cell wall formation and, hence, overall growth and development processes.

### THE SPACECRAFT AS A BIOCHEMISTRY/MOLECULAR BIOLOGY LABORATORY

The last 25 to 30 years of spaceflight research has allowed scientists to begin to recognize the research potential of carrying out experiments where the gravity vector has been removed. Many of these experiments have, as stated earlier, given interesting phenomenological observations, which still await clarification at the biochemical and genetic levels. But spaceflight research has been technologically limited in terms of carrying out the best experiments in space biology. These limitations are apparent even today in the experiments designed for SSF. As recently as 1989, NASA designated several areas for investigation in space, and they reflect the need for developing simple growth parameter conditions before more sophisticated research projects can be undertaken (Johnson et al., 1989). These studies include, for example, optimization of plant nutrient and water supplies and plant holding facilities; the ability to grow multiple generations of organisms; determining the effects of microgravity on gas exchange; the control of development at organ and cellular level in microgravity; and other experiments to establish baselines of capabilities. All were included to determine the limitations of basic growth and development processes in microgravity, and all reflect our inability to carry out even the simplest biochemical and molecular biological experiments.

Thus, given the very short time frame to the launch of SSF, the research programs to be included in the SSF agenda must be selected in the immediate future to ensure that the *most modern, highest quality science* is conducted. We recommend that the following be undertaken: (1) rapidly define and design all basic experimental equipment needed for con-

ducting space biology experiments, which is currently under way; and (2) define 8-12 key experiments (or questions) that need to be answered in microgravity for each discipline, and identify and assemble *teams* of investigators (inter- and multi-disciplinary) who have the ability to use today's technologies and today's ideas to address segments thereof. This will ensure that the best science will be undertaken and completed and that the potential of microgravity will be realized.

### CONCLUDING REMARKS

The availability of SSF provides an unprecedented and exciting opportunity to systematically determine how gravity affects the growth and development of all life forms. Although a particular emphasis was placed, in the preceding sections, upon plant systems that show fairly unique gravitropic responses, fascinating differences are also noted with mammalian systems (and other organisms) in microgravity. Hence, molecular biological and biochemical studies can be anticipated to yield important information on a variety of subject areas, such as bone formation and structure, immunology, muscle formation, and the cardiovascular system.

This paper focussed on plants, which represent our principal source of food, clothing, shelter, and medicinal compounds. A systematic examination of the effects of gravity on plant growth and development in the absence of gravity, at the genetic/biochemical level, will allow us to identify and design new ways to biotechnologically exploit plant life in a manner hitherto not possible. It can be anticipated that this will greatly assist in resolving numerous outstanding technical questions, including finding better ways to produce foodstuffs, enhancing the production of medicinals, and improving the supply and quality of wood and related fibrous materials for future generations.

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