AN UPDATE ON PLANT SPACE BIOLOGY

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ABSTRACT

Space craft in low-Earth orbit can provide a microgravity laboratory for the study of the fundamental processes underlying gravity perception and plant development. This microgravity environment also presents significant challenges for the cultivation of plants for biological life support systems and as a supplemental food source for astronauts. The goal of this brief review is to highlight the progress made in these areas over the last decade. Although some advances have been made in a number of fields of fundamental space biology, most remain in the early stages of development due to the limited number of opportunities for spaceflight experiments. In almost all research areas, interpretation of flight experiments would be improved through the use of an onboard centrifuge to provide a 1-g control, a capability available on some facilities on the International Space Station (ISS). Paradoxically, just as the ISS nears completion, investment in fundamental biological research and access to this state-of-the-art platform for research in microgravity will become severely limited due to both the retirement of the U.S. Space Shuttle and the reorganization of NASA around the exploration agenda.

INTRODUCTION

Gravity has supplied a constant input throughout the evolution of life on Earth, providing a directional cue by which plants organize cells, tissues, and organs and elaborate their body plans. The various means by which the force of acceleration due to gravity is perceived, transduced, and transmitted throughout the body of the plant remains an active and important research enterprise, drawing upon the latest tools in cell biology, molecular genetics, biochemistry, signal transduction, and physiology to advance our understanding of this complex response (Kiss 2000, Blancaflor and Masson 2003). In addition, the development of an international effort to explore space has provided opportunities to investigate plant growth responses in the microgravity environment of low-Earth orbit aboard Spacelab, Mir, the International Space Station (ISS), U.S. Space Shuttle missions, and various satellite-based lab environments (Ferl et al. 2002, Perbal and Driss-Ecole 2003, Brinckmann 2005). One goal of this review is to focus on the progress made throughout the past decade in understanding the fundamental biological processes through the use of such opportunities.

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A related research enterprise focusing on the utility of plants for performing ecosystem services such as atmospheric O_2 regeneration, water purification through evaporation, transpiration, and food production through photosynthetic energy transduction has also developed as a consequence of the human exploration of space and in response to the possibility of long-term human spaceflight to destinations more distant than the Moon (Ferl et al. 2002, Massa et al. 2006). This enterprise includes the exploration of fundamental biological questions related to the growth and development of plants throughout an entire life cycle in microgravity, resulting in the production of viable seed useful for consumption as a supplementary food source and for the continued cycling of the crop (Kuang et al. 2005). In addition, this enterprise has also required the identification and remediation of the technical barriers associated with growing plants in microgravity. Another goal of this review is to focus on the progress made in applied as well as basic plant research.

Molecular & Cellular Responses

Cell Cycle

Little is known about the influence of gravity on progression through the cell cycle in plants, with some previous reports indicating a decreased mitotic index for plants in microgravity (e.g. Driss-Ecole et al. 1994). This earlier observation has more recently been confirmed by image analysis of nuclear-specific staining of cortical cells in intact roots of lentil (Yu et al. 1999). The observed reduction in the rate of progression through the cell cycle was not accompanied by any change in overall root length, but the authors observed anomalous results in the onboard 1 g centrifuge control, for which the mitotic index was significantly greater than the ground-based 1 g control. These authors speculated that the centrifugation treatment might be providing an additional confounding variable. More recently, in a space experiment, root meristematic cells in microgravity were shown to have an enhanced proliferating rate, but ribosome biogenesis was reduced, as inferred from the nucleolar size and from the levels of the nucleolar protein nucleolin (Matía et al. 2005).

Signaling within a single-celled model system

A number of single-celled model systems exist for studying the earliest events in gravity signal transduction in plants. The great advantage of such a system is that both gravity sensing and response happen within the single cell, eliminating the complexity inherent in multicellular organisms in which the signal may be perceived in one location and transmitted to a separate

location. One such model system is the spore of the fern *Ceratopteris richardii*, in which the site of rhizoid emergence is predicted by the migration of the nucleus in response to gravity (Edwards and Roux 1994). In microgravity, the migration of nuclei in fern spores occurred in random directions (Roux et al. 2003). The ensuing cell division occurred normally, but due to the random migration of the nucleus, the asymmetric division resulting in the formation of the rhizoid initial, and hence the location of emergence of the rhizoid, were random.

Another important single-celled system in space biology is the rhizoid of the alga *Chara* (Kiss 1994, Braun and Limbach 2006). Research on the Space Shuttle or in a sounding rocket investigated the role of the actin-myosin system in the position of the gravity-sensing statoliths (Braun et al. 2002). These authors concluded that actomyosin forces act on statoliths in order to keep them in a position where they function as susceptors and initiate gravitropic reorientation. Interestingly, this observation was true even in cells that had never experienced gravity during their growth and development. This group also suggested that gravity perception in rhizoids requires contact of statoliths with membrane-bound receptor molecules rather than pressure exerted by the weight of statoliths (Limbach et al. 2005).

Gene expression changes in microgravity

In addition to cell biological and physiological data, Roux et al. (2003) also isolated RNA from fern spores harvested at various time points throughout the experiment in microgravity and performed differential display RT-PCR. They found preliminary evidence of significant differences in gene expression for single-cell spores developing in microgravity compared to the 1 g control, but were limited by the analytical techniques available at the time. The investigators have more recently utilized microarray technology coupled with quantitative RT-PCR to identify and confirm differences in gene expression between microgravity and 1 g within the spore (Salmi and Roux 2008). They found differential expression of approximately 5% of predicted genes, with microgravity up-regulating some messages and down-regulating others.

The majority of differences in gene expression between plants grown in microgravity and 1 g have focused on representatives of the flowering plants. One approach has been to track the expression levels of individual transcripts of interest that have a known or predicted role that is expected to change during exposure to microgravity. For example, the expression of a cucumber homolog of the IAA1 early auxin up-regulated gene, Cs-IAA1, was probed using in-situ mRNA localization (Kamada et al. 2000). Expression of Cs-IAA1 served as a biomarker for differential auxin transport because it is induced in the presence of IAA; the authors reported in this case the lack of auxin redistribution in microgravity, implying a link between gravity perception and auxin transport. Expression of alcohol dehydrogenase (ADH) genes has also been targeted, mainly due to the potential for the root zone to become hypoxic in microgravity (Porterfield et al. 1997). The induced expression of ADH genes has proven useful in the construction of a plant biosensor for hypoxic stress by fusing an ADH promoter to the chromogenic reporter β-glucuronidase (Paul et al. 2001, 2002) or to the green fluorescent protein. In the case of the latter, it becomes possible to perform remote live plant imaging, which may have important applications for the growth of plants on future flight missions (Paul et al. 2008).

In addition to the study of individual genes, the advent of cDNA microarray technology has made it possible to survey genome-wide changes in gene expression for plants grown in microgravity and exposed to various environmental conditions in the absence of 1 g. One such study reported on the differential expression patterns observed for Arabidopsis plants on board Space Shuttle mission STS-93. Paul et al. (2005) reported that a number of genes related to heat shock were induced in microgravity despite the lack of a significant temperature stress occurring on flight. The authors suggest that factors other than temperature may also regulate the expression of these genes in an unknown fashion. Another experiment utilizing Arabidopsis microarrays has reported the differential regulation of hundreds of genes upon exposure to light in microgravity compared with the 1 g control (Stimpson et al. 2009), but these analyses are ongoing and additional details are forthcoming (Kiss et al. 2009).

Cell Wall Development

As an acceleration force, gravity may act to guide plant morphogenesis through the highly regulated process of cell wall biosynthesis and subsequent lignification (Scheible and Pauly 2004). Alterations in the oriented deposition of cellulose microfibrils, the synthesis of various cell wall carbohydrates, or the complex process of lignification represent possible pathways through which the plant may regulate mechanical reinforcement under varying gravitational loads.

The properties of cell walls formed in microgravity have been investigated recently, and several patterns are beginning to emerge. Exposure to microgravity appears to reduce the thickness of the cell wall (Hoson et al. 2002, Hoson et al. 2003, Soga et al. 2002). This cell wall thinning may be attributed to the degradation of various cell wall constituents, including a breakdown of xyloglucans in the Arabidopsis hypocotyl (Soga et al. 2002) or other wall hemicelluloses in the rice coleoptile (Hoson et al. 2002). In rice roots, Hoson et al. (2003) found a decrease in both cellulose and matrix polysaccharides and a slight increase in hemicelluloses. This group also reported an increased wall extensibility that correlated with an increased rate of elongation growth in microgravity, except in the case of roots, which showed a slight reduction in wall extensibility despite the increased growth rate. In contrast, in another space experiment with potato tubers, the authors found that there was no difference in cell wall pectins between

space-grown and Earth-grown plants (Cook and Croxdale 2003).

With regard to cellulose microfibril orientation in microgravity, DeMicco et al. (2008) have shown that microfibrils of soybean primary xylem vessels lack orientation when deposited in microgravity, but that secondary walls show normal microfibril orientation in microgravity. This is in contrast to the findings of Levine et al. (2001), which showed no difference in wheat root cortical parenchyma cell wall ultrastructure between microgravity and ground-based treatments.

Growth Responses

Gravitropic sensing and response

The use of the microgravity environment of space craft in low-Earth orbit over the last decade has yielded important new insights into how plants sense and respond to gravity. In particular, utilization of an onboard centrifuge to apply a known acceleration to growing plants allowed Driss-Ecole et al. (2008) to estimate that the threshold acceleration perceived by roots in microgravity was 1.4×10^{-5} g, and a gravity dose of less than 1 g × s. Such low values for both of these parameters requires, from a thermodynamic standpoint, that other forces are at work to displace statoliths, a role the authors propose to be played by the actomyosin network, which they have previously shown to exert a force approximately 1/7 that of gravity on the statoliths (Driss-Ecole et al. 2000). The interpretation of these values is also complicated by the fact that gravity sensitivity may increase when plants are grown in microgravity (Perbal et al. 2004).

Kiss et al. (1999) also utilized an onboard centrifuge to quantify the differences in hypocotyl gravitropic competence between mutants intermediate for starch biosynthesis, finding a correlation between the amount of starch present and gravitropic responsiveness at different time points. The absolute rates of response in these experiments were compromised by a high ethylene concentration, leading to a reduction in elongation rates and gravitropism, but significant differences in the response to gravity between wild-type and starch biosynthesis mutants persisted nonetheless. The authors argue that this correlation, which recapitulates the correlation between starch content and gravitropic curvature in hypocotyls in ground-based observations (Kiss et al. 1997), further supports the starch-statolith model of gravity perception. In addition, in these and other space experiments, ethylene aboard space craft may decrease starch content in gravity perceiving statocytes, and decreased starch could account for decreased gravitropic sensitivity of plants grown in space (Guisinger and Kiss 1999).

Endogenous Growth Responses

The microgravity environment has also allowed for the disentanglement of various developmental and endogenous growth responses from the gravity response pathway proper. One example of this has been to test whether either automorphogenesis or autotropism depend upon a gravity cue for their effect. Automorphogenesis, a spontaneous growth response to an endogenous signal, was unequivocally observed in microgravity in pea epicotyls and roots, which resulted in their curvature away from the cotyledons (Ueda et al. 1999). This group also reported a similar response in the *Ageotropum* mutant of pea grown at 1 g, along with a reduction in polar auxin transport in this mutant (Miyamoto et al. 2007), which further supports the conclusion that this endogenous growth response is gravity-independent. A similar curvature of roots away from the cotyledons at the base of the hypocotyl was reported in microgravity for lentil roots (Driss-Ecole et al. 2008). With regard to autotropism, a straightening of a previously curved organ upon withdrawal of the stimulus causing curvature, Stankovic et al. (2001) showed that this response persists in microgravity and suggest that this is a response reflecting a tendency toward organ axiality.

Another apparently endogenous growth movement is circumnutation, the spiral growth pattern traced in three dimensions by an elongating organ. Johnsson et al. (2009) tested whether gravity influenced either the period or amplitude of circumnutation by growing plants in microgravity and exposing them to periods of 1 g by centrifugation. They found that gravity greatly increased the magnitude of circumnutation in the lateral branches of Arabidopsis inflorescence stems. Light also had a dramatic effect on this growth response, primarily by increasing the frequency of circumnutation, but also by increasing its amplitude. Interestingly, the authors reported observing circumnutations in both clockwise and counterclockwise orientations, suggesting the lack of an endogenous regulator of this response. These researchers also showed that endogenous nutations occur in stems of plants that developed in microgravity as Charles Darwin would have predicted but also that gravity (provided by the centrifuge) amplifies these circumnutations.

Phototropism

Among the many environmental cues that shape plant growth and development, the interaction between light and gravity is likely to play a central role in determining plant form (Correll and Kiss 2002, Molas and Kiss 2009). For example, it has been shown that root phototropic responses represent the response to unilateral light as it is modulated by the change in an organ's orientation in the gravitational vector (Vitha et al. 2000; reviewed in Hangarter 1997) and that experiments performed in microgravity could help to separate these overlapping signal transduction pathways. The most definitive experiment performed to date, however, reported only a slight increase in phototropic curvature of wheat coleoptiles in microgravity relative to 1 g controls, and no dramatic difference in the light dose-response curve (Heathcote et al. 1995). A major experiment aimed at characterizing phototropism in both roots and hypocotyls

of Arabidopsis is currently underway, and takes full advantage of the molecular genetic tools available to probe these two signaling pathways (Kiss et al. 2007, 2009). Among other questions, this project aims to provide insight into the recently-characterized positive phototropic response of roots toward red light and mediated by phytochromes A & B (Ruppel et al. 2001, Kiss et al. 2003).

Phototropism toward a unilateral red light source and mediated by phytochrome has also been shown in the moss *Ceratodon*. Kern and Sack (1999) showed that gravity and light interact when the fluence rate of light is less than 140 nmol m^{-2} s^{-1}, but that above this threshold gravitropism was suppressed and phototropism predominated. The reduced gravitropic response was not correlated with reduced statolith sedimentation, but dark-grown cultures that had previously been exposed to red light showed a spiral growth pattern in microgravity, with individual protonemata aligning in clockwise concentric arcs (Kern et al. 2005). The authors propose that this pattern represents a default growth habit that is masked when a gravity cue is present.

Whole-Plant Studies

Embryogenesis & Seed Development

Following pollination, as the embryo begins to develop, it receives nutrition from the parent plant in the form of starch deposited in the seed, much of which is absorbed by the developing embryo and stored as starches, lipids, and proteins. Kuang et al. (2005) reported that the rate of this process of uptake and conversion is reduced in *Brassica* plants grown in microgravity. They observed a preponderance of starch grains persisting in the outer seed layers well beyond that of ground-based control plants, suggesting an overall reduction in the rate of seed and embryo maturation in microgravity. They also reported a reduced deposition of storage proteins and fewer lipid bodies present in microgravity tissues, further substantiating the hypothesis that the tissue was physiologically younger. These results are in agreement with those of Popova et al. (2009), which also showed an increase in the persistence of endosperm starch in lieu of uptake by the developing embryo. In these experiments, hand-pollination was performed during the spaceflight experiment to test whether the reduced embryo formation and seed set in previous experiments was due to some influence of microgravity on fertilization or simply a low rate of pollination. The results strongly support the latter explanation, as the number of seeds per silique were not significantly different than for the ground control (Popova et al. 2009). More broadly, these results support the idea that the environment of the growth facilities in a spaceflight experiment is extremely important for the discrimination of true microgravity effects from other stresses associated with spaceflight (Paul et al. 2004, Correll and Kiss 2008).

One hypothesis to explain why these tissues may be physiologically younger than their Earth-grown counterparts is that their metabolism is being limited due to hypoxia. For example, similar patterns of storage accumulation have been observed in *Brassica* seed development under reduced partial pressures of oxygen (Porterfield et al. 1999). Briarty et al. (2004) reported that seed lipid utilization during germination in microgravity was impaired by approximately 50% relative to ground controls, effecting a reduction in root growth rate, both of which may be explained by localized hypoxic conditions. This explanation is further supported by the argument that the thermodynamic conditions giving rise to convective air flow near the surface of plant tissues is significantly altered in microgravity, leading to an increase in boundary layer resistances and therefore a reduction in gas exchange potential (Porterfield 2002). The limited contribution of convection on gas exchange in most microgravity-based plant growth environments, coupled with the relatively negligible influence of diffusion at the scale of whole tissues and organs, is hypothesized to account for local regions of hypoxia for certain tissues in microgravity. The observations outlined above and those from other spaceflight experiments suggest that the hardware and chambers used to grow plants in space may be suboptimal and that future work needs to be devoted to developing adequate facilities for growing plants in space (Correll and Kiss 2008).

In addition to changes in primary metabolic storage compounds, some evidence now exists that the biosynthesis and storage of secondary compounds may be altered by microgravity. For example, the major class of defense compounds produced by members of the Brassicaceae is the glucosinolates. Levels of glucosinolates *Brassica* stems grown in microgravity increased 75% relative to ground-based controls (Musgrave et al. 2005), which could have significant implications for the flavor profile of plants grown for food on long duration flights.

Photosynthesis & Gas Exchange

There is only weak evidence to date that microgravity causes any alterations to the structures and/or functions of the photosynthetic apparatus. Chlorophyll fluorescence measurements on *Brassica* leaves exposed to microgravity on the Space Shuttle indicated some slight differences between flight and ground samples (Kochubey et al. 2004). The fluorescence spectra suggest a reduction in light harvesting complexes for both PS II and PS I and reduced overall photochemical efficiency for PS I in leaves grown in microgravity. The authors also reported a higher Chl a/b ratio, a result that was also observed by Jiao et al. (2004) for cotyledons of *Brassica* exposed to microgravity. Jiao et al. (2004) used a biochemical approach on tissues exposed to microgravity, finding a reduction in the amount of PS I core complexes and concomitant reduction in PS I photochemistry *in vitro*. In addition, they found fewer oxygen evolving complexes in

tissues from microgravity, although PS II core complex levels were similar to ground tissue levels.

Despite these findings of shifts in levels of key members of the photosynthetic apparatus, the influence of any such changes at the molecular level appear not to influence rates of photosynthesis as measured at the leaf and canopy levels in microgravity. Working with a dwarf wheat cultivar (*Triticum aestivum* L. cv. 'USU Apogee') some corroborating evidence of the aforementioned reduction in electron transport was reported at high light intensities, but no significant differences between microgravity and 1 g tissues were seen in net photosynthesis, photosynthetic photon flux compensation point, quantum yield, or CO_2 compensation point (Stutte et al. 2005). Similarly, measurements of photosynthetic rates using gas exchange techniques for whole canopies growing in microgravity failed to reveal any significant differences in net photosynthesis at saturating CO_2 concentrations (Monje et al. 2005). In analyzing gene expression of over 800 cDNA's from these tissues, no statistically significant differences were found between ground and flight tissues, further substantiating the claim that microgravity is not producing a recognizable change in photosynthetic tissues (Stutte et al. 2006).

CONCLUSION

While progress has been made in a number of the fields related to plant space research highlighted here, it is clear that the various areas of inquiry remain in their infancy. The cost, complexity, and lack of more widespread opportunities for microgravity-based experiments has resulted in the advancement of only a small sampling of important questions from each of these areas.

Many spaceflight experiments have been hampered by poor quality growth units for growing plants and by the lack of a 1-g control (Correll and Kiss 2008). In order for us to move ahead in identifying true microgravity effects and for using microgravity as a unique research tool, it will be important for more space laboratory facilities to incorporate an on-board 1-g centrifuge as an essential control (Guisinger and Kiss 1999, Cook and Croxdale 2003).

A current paradox in space biology research efforts is that just as we are approaching the completion of the ISS in 2010, the access to this extraordinary research laboratory will become more difficult due to the retirement of the Space Shuttle. Transportation of samples to and from the ISS will be extremely limited, especially since down mass will be possible only in cramped Russian space vehicles. This trade-off compromises a decades-long investment in the ISS for scientific research by limiting access to the ISS just as it becomes a robust experimental platform.

A related issue is the low levels of funding for the space biology community due to the new emphasis on developing a new space vehicle for the exploration agenda set forth by the previous presidential administration. However, it is important to remember that the success of a human mission to Mars relies upon a better understanding of microgravity effects on plants and other living organisms, which can only be attained through sustained, long-term investment in fundamental research.

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