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Molecular Aspects of Stress-Gene Regulation During Spaceflight

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Abstract

Spaceflight-associated stress has been the topic of investigation since the first terrestrial organisms were exposed to this unique environment. Organisms that evolved under the selection pressures of earth-normal environments can perceive spaceflight as a stress, either directly because gravity influences an intrinsic biological process, or indirectly because of secondary effects imparted by spaceflight upon environmental conditions. Different organisms and even different organs within an organism adapt to a spaceflight environment with a diversity of tactics. Plants are keenly sensitive to gravity for directed development, and are also sensitive to other stresses associated with closed-system spaceflight environments. Within the past decade, the tools of molecular biology have begun to provide a sophisticated evaluation of spaceflight-associated stress and the genetic responses that accompany metabolic adaptation to spaceflight.

Introduction

Plants are well suited to the investigation of the effects of a novel stress—like spaceflight—as the sessile nature of plants requires that plants deal with stress by adaptation *in situ* rather than by the active avoidance response that is available to more motile creatures such as animals. Plants have evolved to deal with stress by responding with changes in metabolism to meet the challenge (Aon and others 2000; Bohnert and Sheveleva 1998). Adaptive pathways are common in plants, especially among those living in extreme environments. Yet, as much as a xerophytic cactus may differ in appearance from a grass adapted to an anaerobic salt marsh, both tap a remarkably parallel set of metabolic pathways to achieve the same end—survival in a stressful environment. Even plants that are considered to be stress sensitive, such as *Arabidopsis thaliana* L., are capable of surviving short-term stress by initiating metabolic processes to cope with the new environment (Aon and others 2000; Bohnert and Sheveleva 1998; Daugherty and others 1994). Most significantly, if a plant is challenged with a new environment that does not neatly fall into a well-defined stress category, the plant will often attempt to adapt by utilizing existing pathways that best fit its perception of that stress. Spaceflight is clearly not a stress that plants have earmarked in their toolbox, yet when exposed to it, they utilize existing pathways in mounting a response to deal with this unique environment (Dutcher and others 1994; Halstead and Dutcher 1987; Krikorian and others 1992).

Why is Spaceflight Stressful?

Despite serene images of weightless existence, the spaceflight environment appears to impose stress on plant metabolisms on several fronts (Dutcher and others 1994; Halstead and Dutcher 1987). Basic processes such as gravity perception (Kiss and Edelmann 1999), photosynthesis (Tripathy and others 1996), and reproduction (Musgrave and

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others 1997) are all apparently disrupted to some degree during spaceflight, and there remains much discussion as to the source of those disruptions. Although spaceflight presents a novel environment for terrestrial plants, some of the components of spaceflight-stress are potentially similar to existing earth analogs. For example, spaceflight-limited air convection can contribute to localized hypoxia (Porterfield and others 1997b) and to locally high concentrations of gasses such as ethylene (Guisinger and Kiss 1999; Kiss and others 2000b; Salisbury 1999). Both of these convection-related effects were among the first plant stresses associated with spaceflight to be intensively investigated, and both of these stresses have well-characterized terrestrial analogs.

Hypoxic stress is a common experience in plants that undergo flooding. Partly because of the potential economic impact of flood induced farm losses, the hypoxic response has been studied for a number of years. In general, plants utilize a variety of mechanisms to deal with hypoxia, emanating from the levels of whole plant physiology (ap Res and others 1987; Crawford 1982; Drew and others 2000; Porterfield and others 1997a; Vartapetian 1991; Vartapetian and Jackson 1997) and molecular cell biology (Chang and others 2000; de Vetten and Ferl 1995; Dennis and others 2000; Dolferus and others 1994; Mitchell and others 1984; Papaseit and others 2000; Paul and Ferl 1991, 1997). Data from several experiments included observations that spaceflight plants showed characteristics similar to those of plants experiencing hypoxic stress in terrestrial models (Paul and others 2001; Porterfield and others 2000; Porterfield and others 1997b; Slocum and others 1984; Stout and others 2001). This suggested that a lack of convective air circulation could produce root-zone hypoxia in certain growth media during spaceflight experiments. Plants growing with roots embedded in an agar medium are particularly sensitive, as diffusion of oxygen is further impaired by the surrounding substrate (Chung and Ferl 1999). In such agar-grown plants, spaceflight is associated with an increase in aerenchyma tissue and elevated levels of the enzymes of anaerobic metabolism, both hallmarks of hypoxic stress (Porterfield and others 2000; Porterfield and others 1997b; Stout and others 2001).

Plants naturally produce ethylene as a course of development (Ecker 1995; Wheeler and others 1996). The impact of naturally occurring amounts of ethylene is usually not problematic, but as levels increase, ethylene induces various aspects of maturation and senescence (Solano and Ecker 1998). In a microgravity environment, native ethylene is not readily removed by convection. Localized concentrations of the gas can cause disruptions in cues for reproduction, development, cellular senescence, and starch metabolism (Guisinger and Kiss 1999; Kiss and others 2000b; Klymchuk and others 1999; Madlung and others 1999; Nedukha and others 1999). To further compound this problem, many man-made synthetic compounds outgas ethylene, and ethylene-like molecules. Ethylene can even be introduced into the spaceflight environment by the simple act of crew resupply with fresh fruit. In a closed environment such as an orbital habitat, ethylene can rise to developmentally imperative or phytotoxic levels. For instance, in space station *Mir,* ethylene levels have been measured at over 1000 ppb, a tremendous concentration when compared with a value of 1 ppb in the field (Bugbee 1999). Many of the problems seen in spaceflight experiments in flowering and seed set were eventually linked to elevations in ethylene (Bugbee 1999; Levinskikh and others 2000; Salisbury 1997, 1999).

There is, of course, one spaceflight stress that has no direct correlation with any terrestrial stress. The lack of gravity is the most obvious novel component of spaceflight and it is totally foreign to terrestrial plants. Lack of gravity appears to disrupt developmental cues. Gravitropism is important to plant growth, so it is not surprising that plants have specialized mechanisms to detect and respond to gravity (Bjorkmann 1988; Ingber 1999; Kiss 2000a; Rosen and others 1999; Sievers and others 1996). There are two major ideas as to how plants perceive gravity at the cellular level. The statolith model suggests that sedimentation of subcellular particles is the major contributor to gravity sensing (reviewed in Kiss 2000a), while the gravitational pressure theory suggests that the entire protoplast acts as the gravity sensor (Staves 1997; Staves and others 1997; Wayne and Staves 1997). Both theories rely on gravity exerting a force on cells or subcellular components to elicit gravitropic responses. Currently, the statolith model is the more widely accepted. However, experiments with *Arabidopsis* mutants deficient in starch metabolism indicate that although there is a positive correlation between starch concentration and the ability to respond to gravity, amyloplasts are not an absolute requirement for gravimetric sensing in plants (Kiss 2000a; Kiss and Edelmann 1999; Kiss and others 1999; Kiss and others 1998; Kiss and Sack 1990). Thus, it is likely that additional layers of signaling complexity are involved in the sensing and transduction of gravity signals (Staehelin and others 2000; Weise and Kiss 1999).

A possible contributor to the complexity of gravity sensing and signal transduction may be the in-

volvement of calcium ions (Davies and others 1999; Sievers and others 1996). Theories concerning the role of gravity in calcium-mediated processes include a disruption of the asymmetric redistribution of auxin and calcium across the root tip (Masson 1995) and a disruption in the physical movement of amyloplasts, which purportedly elicits a release of calcium from the Endoplasmic reticulum that ultimately contributes to the lateral polarization of the tissues (Merkys and Darginaviciene 1997). Other studies suggest that although the role of calcium redistribution in the gravitropic response is largely circumstantial (Sinclair and Trewavas 1997), its role in the touch response may indirectly impact gravity sensing (Legue and others 1997). Touch stimulation has long been related to changes in calcium flux, and in *Arabidopsis*, the family of touch-induced genes (TCH) encode calmodulin (TCH1) and calmodulin-related proteins (TCH2, TCH3) (Braam 1992; Sistrunk and others 1994). Cells and tissues that experience increased internal stress, such as root tips, root/shoot junctions, and branch points exhibit elevated TCH3 expression levels, and there is evidence that TCH genes may contribute to cell wall biogenesis. Thus, TCH genes may play a role in the calcium-mediated regulation of changes in cell strength and flexibility (Braam and others 1997; Sistrunk and others 1994), changes that would be seriously mitigated in a microgravity environment. Evidence that cell wall metabolism might be affected by microgravity also includes implications that lignin biosynthesis is repressed in microgravity environments (Cowles and others 1994; Sato and others 1999). Calcium flux may also be disrupted by something as simple as the lighting format in the growth chambers. The 24 hour light regime that is often used in spaceflight experiments can induce a deficiency in species that normally re-supply calcium during dark-period guttation (Bugbee 1999).

The direct effects of altered gravity perception and signal transduction, coupled with the indirect effects of the microgravity spaceflight environment, would seem to offer multiple adaptive challenges and opportunities. The experimental tasks are, therefore, to reveal the underlying responses taking place in order to generate information to enhance the responses or mitigate their cause.

How to Study Adaptive Responses to Stress

The effects of spaceflight on plant growth and development have been evaluated with a variety of approaches, each contributing interconnected data that merge to describe the effects imposed by this environment. The scope of observational data can be extended with a physiological context, and the underlying metabolic processes are in turn supported by a molecular framework that contributes to how a stress response is perceived and delivered to the appropriate organ in the plant. Thus, there are two basic categories of approach: those analyses that profile visual characteristics, and those that profile change on the metabolic or molecular level. The former category includes observing morphological changes in growth habit (roots and shoot orientation) (Hilaire and others 1996; Kiss and others 1999), in pollination and set seed (Kuang and others 1995; Levinskikh and others 2000; Musgrave and others 1997), in cellular organization and ultrastructure (Guisinger and Kiss 1999; Kuang and others 2000; Stout and others 2001; Takahashi and others 2000) and in mitosis and the structure and organization of chromosomes (Facius and others 1994; Krikorian and others 1992; Sytnik and others 1983). The latter category includes the profiling of metabolic proteins and biochemical indicators (Gao and others 2000; Kuang and others 1996; Porterfield and others 1997b; Stout and others 2001; Tripathy and others 1996) and of changes in gene expression patterns (Hampp and others 1997; Kamada and others 2000; Paul and others 2001; Porterfield and others 1997b; Sato and others 1999; Takahashi and others 2000).

Typically, many analyses will include evaluations based on a combination of these approaches, and this is easily evidenced by a quick look at the truncated list of experiments referenced above. Here we will focus on molecular approaches, and discuss the application of some of the tools that have recently come to bear on the question of spaceflight-associated stress in plants.

Analyses of Gene Expression

Analysis of Endogenous Gene Expression

Traditional methods of mRNA analysis can accurately evaluate gene expression levels in whole plants or organs. Specific genes can be evaluated through the use of electrophoresis and hybridization with known DNA fragments as probes (northerns), ribonuclease protection assays (RPA), or Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). In spaceflight applications, materials that have been harvested during flight or shortly after flight would be compared to similar material harvested from ground control plants that were grown under conditions that terrestrially simulate

the spaceflight. The harvested plants or organs would then be ground and the mRNA would be extracted for analysis. These types of gene-specific evaluations have been conducted on spaceflight material from both animals (Brooks and others 2000; Carmeliet and others 1998; Cubano and Lewis 2001) and plants (Kamada and others 2000; Porterfield and others 1997b; Takahashi and others 2000). Since alcohol dehydrogenase (Adh) is a key enzyme in the adaptation to hypoxia, its presence in tissues is taken as a hallmark of hypoxic environments. Porterfield and others (1997b) used RPAs to show an apparent 2-fold increase in Adh mRNA levels in spaceflight plants over the ground-based controls, and this increase was accompanied by an 89% increase in Adh enzyme levels.

Northerns, RPAs, and RT-PCR can be performed in accurate and quantitative fashions. But as powerful as these methods are, they have limitations that directly influence subsequent interpretation. The first limitation is that these methods absolutely depend on a preexisting hypothesis as to which genes to examine, so that the proper probes or primers can be developed. But what about changes in gene expression that might not have been anticipated? As a plant initiates a stress metabolism, whole new suites of genes may be induced, while many others may be silenced. Profiling global changes in gene expression can provide valuable insight into how a plant adapts its metabolism to deal with an imposed stress.

The use of DNA microarrays presents a large-scale method for evaluating thousands of genes simultaneously in comprehensive study of differential expression. Discussion and proposals abound with the potential for using arrays to study spaceflight material. In animal systems this technology has been utilized to evaluate differential gene expression in a cultured human renal cell suspension line (Hammond and others 2000) and in a human leukemic T lymphocyte line (Lewis and others 2001). The renal cells grown by Hammond and others (2000) were cultured in microgravity, hypergravity (3 g), and a rotating wall vessel (RWV) and compared to a static culture grown at 1 g. A noteworthy perspective of this experiment is that the metabolic data suggest that each group of cell cultures grew at a similar rate, indicating no significant differences among the treatments, while the gene array data provide a different view. Of the 10,000 genes surveyed, more than 1,600 showed at least a 3-fold change in expression levels (either up or down) when the microgravity treatment was compared to the ground control. Many of these genes are transcription factors, signaling or receptor proteins, or differentiation mediators. Others play a role in adhesion properties or the cytoskeleton, and a smaller category seems to be composed of genes related to stress, apoptosis and tumor suppression. The T-lymphocytes used by Lewis and others (2001) provide a slightly different perspective. In this case, there were metabolic indicators that spaceflight had an effect on the growth of these cells. T-cell growth is arrested during spaceflight, and the microarray analyses were used to aid in the identification of factors that might contribute to this phenomenon. The list of differentially expressed genes for the T-cells has many parallels to the renal cultures in that prominent classes of genes also included those that regulated transcription, signal transduction, adhesion and the cytoskeleton, tumor suppression and apoptosis. For T-cells, features of the cytoskeleton appeared to be the key to the growth anomalies that were observed in the flight samples, as many of the cytoskeletal elements were not properly reassociating during spaceflight. Disruption in microtubule assembly and organization has been implicated in other spaceflight experiments as well (Boonstra 1999; Krikorian and others 1992; Rijken and others 1994).

To date no published data exist on the use of arrays in plant spaceflight analyses, but emerging DNA microarray data from the BRIC-11 experiments on shuttle mission STS-93 do indicate that young *Arabidopsis* seedlings differentially activate several signal-related genes during spaceflight (A. Reddy, personal communication).

As compelling as these techniques for analyzing native gene expression are, there are common limitations to bulk RNA analyses. The major limitation to these traditional molecular analyses is that the amount of plant material required to isolate sufficient quantities of RNA for arrays, protection assays, and northerns requires that some numbers of plants or plant parts be combined into a single sample. Therefore, the resolution of the analysis is then limited to whole plants, or maybe organ systems at best, and essentially no cell or tissue-specific data on gene expression levels can be derived. One method that can be used to allow tissue and cell specific expression detection is *in situ* mRNA hybridizations. They give excellent cell-specific localization of mRNA populations, but require intense sample preparation. Recently, a spaceflight experiment with cucumber seedlings evaluated the cell-specific differential expression of an auxin-regulated gene, CS-IAA1, between space flight and ground control plants. *In situ* hybridization of CS-IAA1 mRNAs in seedlings demonstrated that auxin does not appear to redistribute in microgravity (Kamada

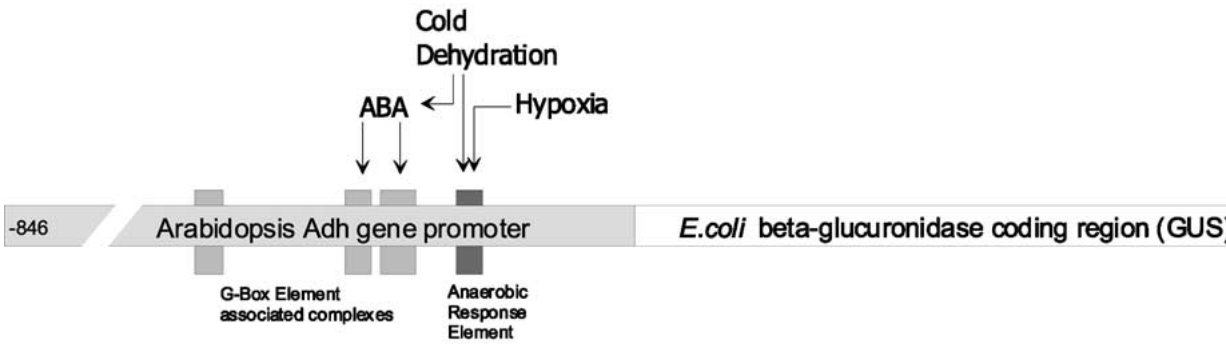

Figure 1. The Adh/GUS reporter transgene. The Adh/GUS reporter is composed of the promoter region of *Arabidopsis* alcohol dehydrogenase (Adh) and the coding region of *E. coli* β-glucuronidase (GUS). The Adh promoter contains a variety of sequence elements that function in the sensing of cold, dehydration, ABA and hypoxia.

and others 2000; Takahashi and others 2000). Another method that can be used to address cell, tissue and organ-specific expression is the use of reporter gene systems that link well-characterized regulatory gene elements with easily detectable gene products that will be present only when the reporter gene is transcriptionally activated.

Analysis of Reporter Gene Expression

The use of reporter genes and transgenic plants offers analytical resolution *in vivo* that is specific to the cellular level, and complements and extends traditional methods of examining gene expression. Reporter gene constructions have been used for many years to characterize the regulatory region of genes and to define the genetic elements that respond to specific signals. A transgene reporter system is constructed by combining the regulatory portion of the gene of interest to the coding region of a gene that provides some sort of clear indicator of expression (Figure 1). That is, when the gene is turned on, the pattern of expression is manifest as deposition of the products of the reporter gene. The deposition pattern of a gene product can provide a very precise evaluation of the localization of gene expression within cells, tissues, and organs of a plant. For instance, direct distinctions can be made between expression in epidermal, mesophyll, or stele cells within a single organ in the plant. All reporter genes are based on the premise that the sensing portion of the engineered gene—the combination of DNA elements that are often referred to as the gene's promoter or transcriptional regulatory region—behaves in a carefully characterized manner. Figure 1 demonstrates the level of detail that can accompany reporter gene constructions, in that well-characterized DNA regulatory elements can be captured and used to detect the activation of specific and well-characterized stress response pathways. The transgene need not fully reflect the native gene expression to provide insightful information. Indeed, reporter gene regulatory units may be intentionally truncated or mutated in order to fine-tune the response of the reporter based on knowledge of the regulatory components. Further, designer promoters can be constructed from regulatory elements from disparate genes in order to create sensors tailored to meet specific environmental requirements. Thus, the regulatory portions of reporter transgenes must be operationally characterized. This means that there is a significant investment in the engineering and design of the reporter transgene, and suggests that there be compelling hypotheses to drive that design. Once characterized, however, reporter transgenes make extremely effective tools for studying gene regulation at cell-specific resolution.

One of the most common protein-coding sequences used in plant reporter gene constructions is the coding region of the *E. coli* β-glucuronidase (GUS) gene (Jefferson and others 1987). GUS has enjoyed extensive utilization by the plant molecular biology community for the characterization of transgene activity. Several features of GUS make it a powerful tool for studying gene expression pattern in plants. First, plants do not have a native equivalent of GUS, which assures that any incidence of GUS expression in a transgenic line is due to the reporter transgene. Second, the transgenic expression of GUS, even in large amounts, does not seem to have a deleterious effect on the host metabolism. Third, the product of the GUS enzyme is very stable, so that tissue expressing GUS can be easily fixed and treated for microscopic inspection (Jefferson and others 1987). There is, however, a negative aspect of GUS as a reporter gene. The stain for GUS activity is not a vital stain, so to visualize GUS expression

plants must be harvested and incubated with the substrate. While GUS activity is clearly revealed by the precipitated stain, the plant is fixed in the process. This single drawback has spurred interest in Green Fluorescent Protein (GFP) from the jellyfish *Aequorea aequorea* (Chiu and others 1996; Haseloff and Amos 1995; Sheen and others 1995). GFP is a vital reporter in that it can be visualized without harvesting the plant, allowing *in vivo* visual inspection of transgene expression in the host plants (Manak and others 2002).

Calibrations of an Adh/GUS Reporter Gene System

Adh/GUS transgenes have been used extensively to evaluate stress metabolism in *Arabidopsis*. The Adh/GUS transgene used in experiment PGIM-01 on STS-93 was constructed from 846bp of the *Arabidopsis* Adh promoter region linked to the coding region of GUS (Figure 1). The reporter gene construction was then transferred into the appropriate vectors for transformation into *Arabidopsis* (Paul and others 2001). The overall response of the Adh/GUS reporter to hypoxic stress is consistent with the native Adh gene. The *Arabidopsis* Adh promoter region is extremely well characterized, and experiments profiling Adh/GUS reporter gene product activity relative to endogenous Adh gene activity show that the two profiles are virtually identical with regard to induction signals and relative amounts (Chung and Ferl 1999). The Adh/GUS transgene is exquisitely sensitive to hypoxia, as well as other stresses (de Bruxelles and others 1996; Dolferus and others 1994; Paul and others 2001).

To calibrate the response of the Adh/GUS reporter stain against the responses to hypoxic conditions, Adh/GUS plants were exposed to varying levels of root-zone flooding and hypoxia. The plants were grown for 9 days on agar plates in a vertical orientation, to keep the roots out of the medium and present a fully aerobic root environment. After the 9-day initial growth, plates were placed in a 2% O_2/98% N_2 chamber for 24 hours, or in a flooding chamber such that either the entire root was flooded to the root/shoot junction or only the more distal portions of the roots were flooded (full or partial flood, Figure 2). The 2% O_2 environment presents whole plant hypoxia, and the root floodings leave the shoot fully aerobic while presenting a relatively mild, root tip hypoxia or more severe full root hypoxia. After the hypoxic induction period, plants on the plates were harvested. Several were placed in GUS stain to evaluate transgene activity and the bulk of the plants were cut at the root/shoot junction to assay Adh mRNA levels in roots and shoots using highly quantitative real-time RT-PCR. As expected, the more severe the hypoxic stress, the more Adh mRNA is transcribed and the more blue Adh/GUS transgene activity is detected (Figure 2). The shoots of plants from 2% O_2 show a more than 10-fold increase in Adh mRNA, and extensive staining of portions of the leaves. The shoots of plants with fully flooded roots show a small, but significant increase in Adh mRNA levels of about 3-fold. The shoots of plants with only the distal portions of their roots flooded do not show any increase in Adh mRNA levels. The roots show a much more dramatic response. Roots from plants in 2% O_2 demonstrate more than 100-fold increase in Adh mRNA, and Adh/GUS transgene activity is observed throughout the roots. Fully flooded roots show an increase in Adh mRNA levels of more than 700-fold and intense Adh/GUS transgene activity throughout the root. Roots that were flooded only in the distal portions demonstrate Adh/Gus staining near the tips only, which is basically consistent with the fact that much of the root was out of the water. While the staining in those mildly hypoxic root tips is quite intense when compared with the control, the overall Adh mRNA levels (when amortized over the whole root that was used for RNA isolation) in these roots rose by only about 15-fold, which is much reduced over the 700-fold induction of the fully flooded roots.

While the Adh/GUS expression patterns of each plant reflect the overall levels of induction, illustrated by the Adh mRNA levels, it is easy to see how the cell-specific nuances of that expression can be lost when whole plant parts are simply ground up and biochemically assayed. The control plants in Figure 2 illustrate that the Adh/GUS expression patterns in a well-aerated plant growing on the surface of vertical plate media are minimal, and this feature is reflected in the quantitative Adh mRNA data. The plants that were exposed to a 2% O_2/98% N_2 atmosphere or flooded up to the root/shoot junction express Adh/GUS at a high level, but the distribution of GUS expression suggests that the two forms of stress evoke different patterns of that expression in the plant. For instance, plants exposed to 2% O_2 atmospheres show extensive Adh/GUS expression in the aerial portion of the plant, while in the flooded plants, leaf expression is usually limited to the shoot apex region. The specificity of this effect is most dramatically illustrated in the plants with partially submerged roots. The Adh/GUS expression in these plants is typically seen only in the distal portions of the root with a concomitant, clear Adh/GUS expression in the shoot apex. However, the

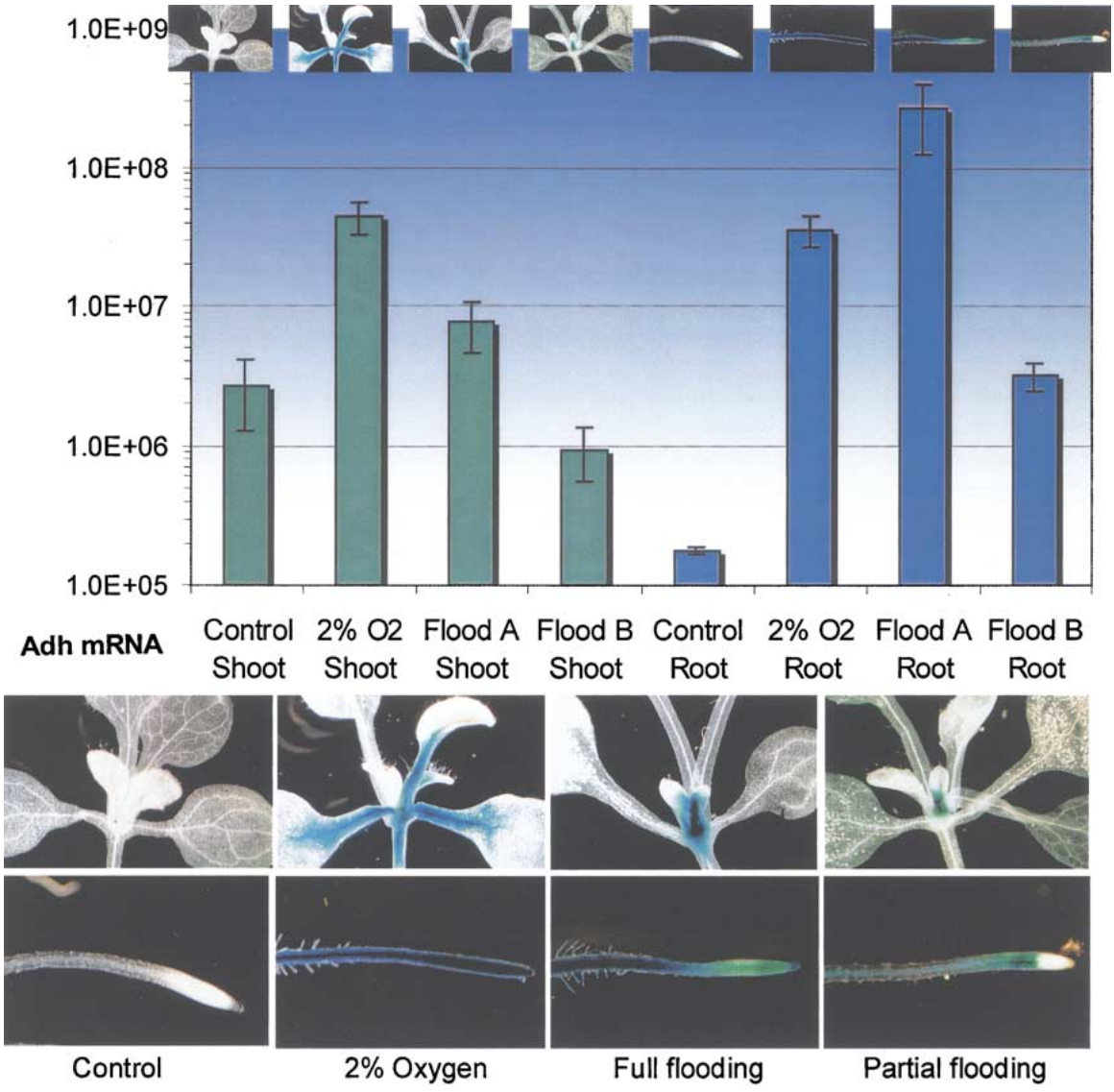

Figure 2. Adh and Adh/GUS expression in transgenic plants. Plants harvested from the controlled inductions were assayed for native Adh gene mRNA levels with quantitative real-time RT-PCR (Taq Man ABI). mRNA levels are displayed as molecules Adh mRNA / equivalent RNA reaction, and are normalized against total RNA used in the reaction. Note that the scale of mRNA levels is logarithmic. The error bars indicate standard deviation from triplicated assays, and all reactions contained an additional internal ribosomal RNA reaction for normalization. The values for shoot tissues are shown in green bars to the left and values for roots are shown in blue bars to the right. Small pictures of representative stained plants are shown above the corresponding bar in the graph. Larger pictures of the stained plants are displayed below the graph, with shoots and roots arranged in columns from left to right: control, 2% O_2, full root flood and partial root flood.

expression in the shoot is so highly localized that simple quantitation of Adh mRNA levels in roots and shoots failed to detect any inductive signals arriving in the shoots.

These types of experiments were conducted with an extensive variety of stresses to calibrate the Adh/GUS transgene expression before, during, and after plants with the Adh/GUS transgene were flown on shuttle mission STS-93 to evaluate spaceflight induction of gene expression (Paul and others 2001). For example, an evaluation of the effects of agar media on root-zone hypoxia led to the realization that while roots tunneling into agar are sufficiently aerated to remain healthy, they were under sufficient hypoxic stress to induce expression of Adh genes. Transgene expression patterns further illustrated that the roots were experiencing localized hypoxic stress and that the stress response signal

was transmitted to the normoxic aerial portion of the plant, where it was manifested as Adh/GUS expression in the shoot meristem. This signal transduction could be interrupted by Ca^{2+} blockers, suggesting that hypoxic stress signal transduction from root to shoot is mediated by a Ca^{2+} requiring pathway (Chung and Ferl 1999; Paul and others 2001).

Transgenic *Arabidopsis* and Spaceflight Signal Transduction

Space Shuttle *Columbia* carried Adh/GUS transgenic plants to investigate the effects of spaceflight on plant metabolism through the specific monitoring of Adh gene expression. The experiment, PGIM-01, was launched on STS-93 in July 1999 for a 5-day mission (Paul and others 2001). As in the calibration tests, the seedlings were grown on the surface of square, nutrient agar plates held in the vertical position to prevent agar-induced expression. Both the ground control and the flight plants maintained a root growth habit along the surface of the medium; the ground controls growing straight down the surface of the plate while the roots of the flight plants wandered randomly across the surface.

The flight plants displayed Adh/GUS expression in the distal region of the primary roots, while the shoot portions of the plants did not express Adh/GUS. The qualitative pattern of expression seen in the space flight plants was not consistent with the patterns of GUS expression in deliberately stressed ground controls designed to mimic root-zone hypoxia. All terrestrial forms of hypoxic stress, even partial root-zone flooding, are accompanied by Adh/GUS expression in the shoots (Figure 2). Controlled induction experiments were conducted on the ground in the presence of agents that disrupt calcium-mediated signal transduction. Plants that were stressed to mimic root zone hypoxia in the presence of gadolinium chloride or ruthenium red were the only plants that displayed Adh/GUS patterns virtually identical to the flight plants (Paul and others 2001). These results suggest that either the normal hypoxia response signaling from the roots to the shoots is impaired in spaceflight or that spaceflight inappropriately induces Adh/GUS activity in the roots for reasons other than hypoxia.

Discussion

The spaceflight environment imposes stress upon terrestrial organisms that may lie completely outside of their evolutionary experience. Organisms appear to cope with novel environmental challenges by adapting existing stress management strategies and inducing suites of genes encoding adaptive metabolic pathways. Due to their sessile habit, plants are masters of metabolic adaptation, and thus are ideally suited to aiding in the dissection of the stress response pathways activated by spaceflight. Understanding stress gene regulation then allows for engineering advances to mitigate the stress and opens the way for bioengineering of adaptive metabolisms, or for making informed choices among existing species, in cases where the stress is an intrinsic biological response.

Investigations into how environmental signals are translated into appropriate responses at the gene expression level are basic to all queries on the effects of stressful environments, and alterations in calcium signaling are often central parts of stress response pathways. It has been suggested that calcium ions may play a role in gravity perception through amyloplast interactions with the cytoskeleton and endoplasmic reticulum (ER) that leads to the release of calcium to modify the activities of enzymes and receptor proteins. A differential flow of calcium and the plant hormone auxin (IAA) is thought to lead to laterally differentiated growth in tissues. The concept that auxin may convey the gravity signal from the sensor cells to the cells targeted for elongation has been considered (Lomax 1997), indirectly supporting the possibility that an interruption of the flow of either auxin or calcium could disrupt a plant's ability to sense a gravity vector. In a spaceflight study with cucumber (Kamada and others 2000), it was concluded that microgravity disrupted the ability of auxin to be redistributed in the hypocotyl/root transition zone. Spaceflight also disrupts the ability of fern spores to establish proper polarity. In ground-based studies, treatments of spores with a calcium-channel blocker that reduced the calcium current similarly disabled the orienting influence of gravity (Chatterjee and others 2000). Chen and others (1999) have compiled an excellent review addressing the role of calcium ions, auxin and the cytoskeleton in the gravity signal transduction pathway.

Signal transduction and calcium may play a direct role in the symptoms observed in spaceflight plants. It has long been observed that plants grown in microgravity environments have many of the features of stressed plants by hypoxia (Paul and others 2001; Porterfield and others 2000; Porterfleld and others 1997b; Slocum and others 1984; Stout and others 2001) and interruption of calcium flux is known to affect the hypoxia response (Subbaiah and others 1994a). Many of the secondary effects of hypoxic stress such as aerenchemya formation and tissue

necrosis also involve calcium signaling (Drew and others 2000; Subbaiah and others 2000; Subbaiah and others 1994b). The PGIM-01 spaceflight experiments using the Adh/GUS transgene also implicate calcium as a target for disruption during spaceflight. The only way to recreate the Adh/GUS expression pattern that was seen in the flight plants in the ground controls was to interrupt calcium-mediated signaling in plants exposed to limited root-zone hypoxia (Paul and others 2001). A spaceflight experiment employing an on-board 1-g centrifuge could address whether a gravity vector can restore these apparent disruptions in signal transduction, an approach that has been employed with success in several plant flight experiments, but one that is not always available (Driss-Ecole and others 1994; Guisinger and Kiss 1999; Heathcote and others 1995; Iversen and others 1996; Kiss 2000a; Kiss and Edelmann 1999; Kiss and others 1998; Legue and others 1996; Papaseit and others 2000; Perbal and others 1987).

Continued analysis of specific gene expression patterns will be necessary to fully resolve the issues of stress perception and signal transduction in plants grown during spaceflight. It is likely that movement toward a complete understanding of the impact of spaceflight on gene expression will require experiments using complementary molecular tools, from DNA arrays for whole plant or organ system responses, through traditional molecular analyses of particular genes and the use of reporter gene constructions for testing specific regulatory hypotheses.

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