MECHANISMS OF GRAVITY PERCEPTION AND TRANSDUCTION IN PLANTS: RESULTS OF GROUND AND SPACE EXPERIMENTS

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Abstract

Gravitropism is directed growth of a plant or plant organ in response to gravity and can be divided into the following temporal sequence: the perception, transduction, and response phases. This paper is a review of the research on gravity perception and transduction mechanisms in plants. The evidence for the two standing theories of perception, the starch statolith and protoplast pressure models, is presented along with a new theory (the statolith pressure) that attempts to synthesize both models. This review considers both ground-based and spaceflight research and ends with results of recent flight experiments that address these issues.

Key words: gravitropism, statolith, *Arabidopsis*, spaceflight experiments, starch-deficient mutant.

Introduction

Plants are capable of very exquisite responses to their environment, and the tropisms provide an excellent example of this phenomenon. A tropism is a directed growth response to an external stimulus such as light (phototropism), touch (thigmotropism), or gravity (gravitropism, formerly known as geotropism). This paper provides a review of plant gravitropism with a focus on the earlier events of the gravitropism pathway, namely the perception and transduction phases. Other recent review articles on this subject include those by SALISBURY, 1993; BALUSKA and HASENSTEIN, 1997; SACK, 1997.

In terms of sensory physiology, gravitropism is the directed growth of a plant or plant organ in response to the force of gravity. The stages of gravitropism in plants can be divided into: perception, transduction, and response (EVANS *et al.*, 1986; SALISBURY, 1993). In roots, gravity perception is hypothesized to occur in the rootcap region, and the response (differential growth) occurs in the zone of elongation (SACK, 1991). In shoots, the endodermis appears to be the site of perception (KISS and SACK, 1990; FUKAKI *et al.*, 1998). In higher plants, there is a cellular and spatial separation between perception and response, and the signal must be transmitted over a relatively large distance (i.e. many cell layers). In certain lower plant groups, all phases of gravitropism occur in a single cell (SIEVERS *et al.*, 1996; KISS, 1997).

Hypotheses for Gravity Perception

Until recently, there have been two principal models for plant gravity perception (SACK, 1997). First, there is the **starch statolith hypothesis**, which proposes that perception is mediated by the interaction of dense organelles (termed statoliths) with other cytoplasmic structures. In higher plants, the statoliths appear to be amyloplasts in specialized cells while other types of sedimenting particles have been found in algae. The statolith model has been supported by work from our group and others (e.g. KISS *et al.*, 1989, 1996, 1997; VITHA *et al.*, 1998). An alternate model is the **protoplast pressure hypothesis**, which has been advocated by Wayne and co-workers (e.g. WAYNE *et al.*, 1992; STAVES *et al.*, 1995, 1997a, 1997b). From a thermodynamic perspective, calculations show that either model is feasible (HASENSTEIN, 1999). However, recently PERBAL (1999) has attempted to reconcile these two models and proposed that both the protoplast and statoliths play a role in perception in his **statolith pressure theory**.

The starch statolith hypothesis has been discussed in numerous older reviews (VOLKMANN and SIEVERS, 1979; EVANS *et al.*, 1986) and more recent publications (SALISBURY, 1993; SACK, 1997). The main lines of evidence for a statolith-based model are presented in these papers and can be summarized as follows. (1) In stems of several plant species, the response to gravity is correlated with the location and extent of amyloplast sedimentation. (2) *Arabidopsis* mutants lacking an endodermis in stems are agravitropic (FUKAKI *et al.*, 1998). (3) During the regeneration of a rootcap following its removal, the ability to perceive gravity was restored when the cap was reformed and new amyloplasts sedimented. (4) Reduced-starch and starchless mutants of *Arabidopsis* and *Nicotiana* have vigorous growth but are less sensitive to gravity in ground-based (KISS *et al.*, 1997; VITHA *et al.*, 1998) and in spaceflight studies (KISS *et al.*, 1998, 1999). (5) Magnetophoretic displacement of amyloplasts induced curvature in roots. (6) Rapid changes in membrane potential occur in central rootcap (columella cells), but not in other cells of the root, following gravistimulation by reorientation.

However, there also is a body of evidence for the protoplast pressure hypothesis (reviewed in SACK, 1997, and STAVES *et al.*, 1997a, 1997b). (1) Sedimenting particles are absent in many gravitatic unicells such as *Euglena* and in internodal cells of the alga *Chara*. (2) The density of external medium alters the gravity-dependent cytoplasmic streaming in internodal cells of *Chara*. (3) There is a possibility (based on inhibitor studies) that integrin-like molecules sense changes in protoplast pressure. (4) The density of external medium also appears to alter gravitropism in roots of higher plant (rice; STAVES *et al.*, 1997b). However, as stated above, a new proposal to integrate both the statolith model and the protoplast pressure model has been made by PERBAL (1999). FURTHERMORE, BARLOW (1995) and SACK (1997) have proposed that several types of gravity sensing exist in plants, although this view has been discounted by the Wayne group (STAVES *et al.*, 1997b).

If statoliths play a role in gravity perception, then there are several possibilities for their mode of action. One view is that sedimentation or (relatively) larger scale movement is needed for statolith function (VOLKMANN and SIEVERS, 1979). In this case, starchless mutants would perceive gravity by another mechanism since it appears that starchless plastids do not move upon reorientation of roots (CASPAR and PICKARD, 1989; KISS *et al.*, 1989; MACCLEERY and KISS, 1997). Another view is that statoliths act through a pressure mechanism and that statolith sedimentation is not need (NICK *et al.*, 1997).

Potential Role of the Cytoskeleton in Gravitropic Signal Transduction

Following the perception of gravity, how is the signal transduced so that a response will occur? Many researchers have suggested the cytoskeleton, both microfilaments and microtubules, plays a role in the perception/transduction phase (BALUSKA and HASENSTEIN, 1997). One of the earliest proposals for microtubulular involvement in gravitropism in plants was by FRIEDRICH and HERTEL (1973). Later studies seem to focus more the microfilament cytoskeleton by a combination of sophisticated immunolocalization studies and by inhibitor studies with cytochalasins (HENSEL, 1985; SIEVERS *et al.*, 1989, 1991). Spaceflight experiments also have examined the interrelationships between statoliths and microfilaments (e.g. BUCHEN *et al.*, 1993). In addition, integrin-like molecules (which may link the microfilaments to the extracellular matrix) in plants have been proposed to be involved in gravitropic signal transduction (WAYNE *et al.*, 1992; KATEMBE *et al.*, 1997).

However, despite the availability of some of the above cited correlative evidence, researchers are now calling into question the role of both microtubules and microfilaments in perception/transduction mechanisms of gravitropism. For instance, microtubules cannot be identified in the tip region of gravitropic *Chara* rhizoids (BRAUN and SIEVERS, 1994). Similarly, microfilament bundles cannot be localized to the central root cap cells of higher plant roots (BALUSKA and HASENSTEIN, 1997). Cytochalasin was shown, in fact, not to inhibit gravitropism in roots of three species of higher plants (STAVES *et al.*, 1997a). In addition, in a recent spaceflight experiment, roots that were treated with cytochalasin and then subject to centrifugation curved *faster* than the controls (PERBAL, 1999)! However, although microtubule arrays and microfilament bundles (i.e., F-actin cables) have been ruled out by most workers, it is possible that single or small groups of microfilaments can play a role in transduction (BALUSKA and HASENSTEIN, 1997).

Starch-Deficient Mutants – Importance in Gravitropism Research

Some of the strongest evidence that amyloplasts function as statoliths comes from our research with starchless mutants of *Arabidopsis* (KISS *et al.*, 1989; SACK and KISS, 1989). Based on detailed studies of the kinetics of gravitropic curvature, the conclusion was that wild-type (WT) roots (with a full complement of starch) are more sensitive to gravity than starchless roots. For example, the presentation time (a measurement of gravitropic sensitivity; JOHNSSON and PICKARD, 1979) was 0.4 minutes for the normal WT and 1.3 minutes for the starchless (TC7) mutant. Other research with *Nicotiana* has shown that roots and hypocotyls of a low starch mutant were much less sensitive to gravity compared to WT roots and hypocotyls (KISS and SACK 1989, 1990; VITHA *et al.*, 1998). In all of these studies, the greatest difference in gravitropic sensitivity was found at threshold „doses" of gravity that were estimated by comparisons of presentation times and perception times.

This work was extended in studies of two reduced-starch mutants and an independently-isolated starchless mutant of *Arabidopsis*, which demonstrated that the degree of graviresponsiveness is proportional to the total mass of plastids per cell (KISS *et al.*, 1996, 1997; WEISE and KISS, 1997). These „intermediate" mutants have 50 and 61% of the WT starch in columella cells of the root cap (KISS et al., 1996) and also are starch-deficient in their hypocotyls (KISS *et al.*, 1997). However, as indicated by STAVES *et al.* (1997b), „while these data are consistent with the statolith theory, they do not discriminate between the gravitational pressure and statolith theories for gravisensing."

Our Spaceflight Experiments with Arabidopsis

Thus, despite extensive study for almost a century, the starch-statolith theory remains controversial, at least to some investigators (HENSEL, 1989). A major reason for this controversy is the intrinsic difficulty of estimating gravitropic sensitivity in a 1-g environment. Since gravity is a constant and ubiquitous force in ground-based studies, the principal method to estimate gravitropic sensitivity (e.g.

presentation time) in plants involves the use of clinostats (e.g. KISS *et al.*, 1989, 1996), which have their own inherent limitations. However, we had the opportunity for a spaceflight project to study gravitropic sensitivity in WT and starch-deficient *Arabidopsis* (two reduced-starch and one starchless mutant) in microgravity by using an in-flight 1-g centrifuge to apply limited amount of gravitational forces so that thresholds could be estimated.

In 1995, our space project was selected by the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA) for definition and development as part of a Space Shuttle payload called Biorack (BRILLOUET and BRINCKMANN, 1997; KATEMBE *et al.*, 1998). The Biorack module is a multiuser facility which serves as a small laboratory for studying cell and developmental biology in unicellular organisms, plants, and small invertebrates (MANIERI *et al.*, 1996). This facility consists of two incubators (each with two variable-g centrifuges), a glovebox, and, during spaceflight missions, a duplicate Biorack unit exists on the ground as a control.

Our first spaceflight project, called PREPLASTID, was a smaller-scale experiment on Space Shuttle STS-81 that flew in January 1997 and was designed to assess the growth/developmental characteristics of the *Arabidopsis* plants (KISS *et al.*, 1998). Based on the PREPLASTID results, we optimized our procedures for the larger-scale PLASTID experiment on STS-84 that flew in May 1997 (KISS *et al.*, 1999).

The most significant result of these spaceflight experiments is that WT hypocotyls of microgravity-grown seedlings had the strongest response to stimuli provided by the 1-g centrifuge while hypocotyls of the starchless mutant did not. The reduced starch mutants exhibited a response intermediate between the WT and the starchless strain.

What was unexpected in our flight studies was the small magnitude of curvature that resulted after the unilateral gravitational stimulus provided by the centrifuge (KISS *et al.*, 1998, 1999). Based on our ground studies (KISS *et al.*, 1996, 1997), we predicted a greater magnitude of curvature for all four strains of *Arabidopsis*. One possible interpretation is that hypocotyls of seedlings grown on the ground are more sensitive to gravity than are those of the microgravity-grown seedlings. These results differ from studies of roots, which have been shown to be more sensitive in microgravity compared to a 1-g environment (PERBAL and DRISS-ÉCOLE, 1994; VOLKMANN and TEWINKEL, 1996; PERBAL *et al.*, 1997). However, since the space grown plants were smaller than the ground controls (KISS *et al.*, 1998), the difference in gravitropic sensitivity may be more related to developmental stage of the seedlings rather than simply differences in sensitivity between roots and hypocotyls.

This experiment on gravitropic sensitivity was performed the „right way" in that brief gravitational stimuli were provided, and the seedlings were allowed to express the response without further unilateral gravity stimuli. Thus, the complications of ground-based experiments performed with clinostats were avoided (SALISBURY, 1993), and the unique environment of microgravity was used to help answer basic questions about biological phenomena (DUTCHER *et al.*, 1994). The results of PLASTID and PREPLASTID support previous ground-based studies of these and other mutants which demonstrate a positive correlation with increasing amounts of starch and increasing sensitivity to gravity. Taken together, the ground and space research are strongly supportive of a statolith-based model for plant gravity perception but still do not exclude the protoplast pressure hypothesis. Further ground-based and spaceflight research should help us to distinguish among the competing hypotheses for graviperception mechanisms in plants.

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