Statolith motions in gravity-perceiving plant cells: does actomyosin counteract gravity?

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ABSTRACT Statocytes from plant root caps are characterized by a polar arrangement of cell organelles and sedimented statoliths. Cortical microtubules and actin microfilaments contribute to development and maintenance of this polarity, whereas the lack of endoplasmic microtubules and prominent bundles of actin microfilaments probably facilitates sedimentation of statoliths. High-resolution video microscopy shows permanent motion of statoliths even when sedimented. After immunofluorescence microscopy using antibodies against actin and myosin II the most prominent labeling was observed at and around sedimented statoliths. Experiments under microgravity have demonstrated that the positioning of statoliths depends on the external gravitational force and on internal forces, probably exerted by the actomyosin complex, and that transformation of the gravistimulus evidently occurs in close vicinity to the statoliths. These results suggest that graviperception occurs dynamically within the cytoplasm via small-distance sedimentation rather than statically at the lowermost site of sedimentation. It is hypothesized that root cap cells are comparing randomized motions with oriented motions of statoliths and thereby perceiving gravity.—Volkmann, D., Baluška, F., Lichtscheidl, I., Driss-Ecole, D., Perbal, G. Statolith motions in gravity-perceiving plant cells: does actomyosin counteract gravity? FASEB J. 13 (Suppl.), S143–S147 (1999)

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Gravity-oriented growth of plant organs such as roots are under the control of at least two signal transduction chains (1, 2). The first of these is located at the site of stimulus transformation, i.e., in statocytes of the root cap (3); the second is at the site of differential growth (4–8) i.e., in cells of the transition zone (9), where a major switch occurs concerning organization and distribution of cytoskeletal elements (9, 10).

In spite of recent controversial discussions of starchless mutants in relation to the first step of stimulus transformation in root cap cells, it is mainly accepted that sedimentable statoliths, starch-filled plastids (amyloplasts), are involved in an intracellular perception mechanism (11). Several sophisticated experiments on clinostats (12) and under microgravity (13) ruled out endoplasmic reticulum (ER) membranes and the plasma membrane as cell structures directly involved in the process of stimulus transformation via membrane contact sites and/or exerted pressure by statoliths. These results, however, did not exclude these membranes as mediators of following steps during signal transduction and transmission. On the other hand, cytoskeletal elements, especially those composed of the actomyosin complexes, came into debate as the structures mediating graviperception (14–18). One of the main findings of these investigations was that the position of statoliths in root cap cells is balanced by two forces, the external force of gravity and the internal tension force exerted by the network of cytoskeletal proteins (15, 16). As such, plant statocytes represent a mechanosensing system comparable to the systems discussed for morphogenetic processes by Ingber’s group under the term tensegrity (19, 20).

This review will focus mainly on microgravity experiments (for recent literature see ref. 21) supporting the idea that stimulus transformation occurs via cytoskeletal proteins measuring randomized versus oriented motions of statoliths.

STATOCYTE POLARITY AND STATOLITHS BEHAVIOR UNDER 1-g CONDITIONS

Random motions of amyloplasts versus oriented motions of statoliths

Root caps are characterized by at least three basic cell types performing different functions: meristem-
atic cells, statocytes that perceive gravity, and secre-
tory cells that facilitate penetration of root apices
through the soil. Thus, the structure and function of
root cap cells change at least twice, which occurs
within a few hours. In meristematic cells, amyloplasts
containing a small amount of starch mainly surround
the nucleus, positioned in the center of the cell. The
main change from the meristematic cell to the
statocyte stage is indicated by cell elongation in the
axial direction accompanied by the development
and establishment of a pronounced cell polarity
where the nucleus is always located at the proximal
pole. On the other hand, ER cisternae are mostly
arranged at the distal pole, whereas sedimented
amyloplasts (density $\rho = 1.44$ g cm$^{-3}$ versus $\rho = 1.03$ g
cm$^{-3}$ of the cytoplasm) are located above the ER and
small vacuoles, dictyosomes, and mitochondria are
located mostly between the two poles (for review see
ref. 22). The development of cell polarity is estab-
lished by cytoskeletal elements, especially cortical
microtubules and actin microfilaments; this has been
demonstrated directly by microscopic approaches
(18, 23) and indirectly after inhibitor experiments
(18, 24, 25). Sedimentation of statoliths is certainly
facilitated by the specific cytoskeletal status of the
statocytes, in so far as endoplasmic microtubules and
bundles of actin microfilaments have not been ob-
served in this type of cap cell (18). However, this
sedimentation is not just a purely physical process
finalized during cell development; it occurs contin-
uously, as shown by the fact that it can be observed in
root cap material from different plants through the
use of high-resolution video microscopy. Statoliths
are continuously in motion, sometimes even showing
saltatory movements (26). Sometimes even motions of single starch grains are visible within
the statoliths (see ref. 28). Thus, in gravity-perceiving
cells random motions of amyloplasts are changed
into directed motions that in summary result in their
sedimentation. In other words, oriented gravity-de-
pendent motions overcome the noise of constitutive
random motions.

**Cytoskeletal elements as a basis for statoliths
motions**

Actomyosin-driven transport of cell structures, espe-
cially chloroplasts, is well documented for algae such as
*Nitella* or *Acetabularia* (29, 30). The latter species
are, due to their large cell size, especially suitable for
injection of marker molecules. With higher plants,
visualization of plant cytoskeletal proteins, especially
of actin and its associated molecules, was hampered
by the lack of adequate methods for a long time.
Affinity fluorescence microscopy using rhodamine-
phalloidin was the preferential approach (for recent
literature see ref. 31), although these investigations
did not take into consideration polymerizing effects
(32) of the fungal toxin on G-actin, and as a conse-
quence the shifting of the ratio between G- and
F-actin toward the filamentous stage. However, spe-
specific antibodies against actin (10, 33) and some associated proteins (34) are now available and a powerful immunohistochemical method has been established (10, 35) for localization of actin and any other plant protein (e.g., see ref. 36). In spite of intensive studies using different immunocytological techniques, visualization of prominent actin microfilaments in root statocytes is still lacking (for recent literature see ref. 17), whereas other cell types of the root proper have been shown to be rich in filamentous actin, distinctly organized in different cell types (10). On the other hand, diffuse actin labeling, especially surrounding the statoliths, suggests high amounts of short F-actin elements and of G-actin in root statocytes. Concerning the motor molecule myosin, essential information is extremely rare for higher plants. Using heterologous antibodies against myosin II from chicken muscle, labeling is prominent only in close vicinity to cress root statoliths (37). Similar observations have been reported for statoliths of maize roots (17) and Chara rhizoids (38). Thus, in root cap statocytes the actomyosin complex seems to be restricted to the periphery of statoliths, enabling on the one hand sedimentation and on the other hand short distance movements. Experiments under microgravity have been performed by our groups showing that the position of statoliths in root cap cells depends on two forces, the external gravitational force and the internal force exerted putatively by cytoskeletal proteins, both acting on the statoliths (16, 25). In these experiments, the possibility of switching from higher g levels to microgravity was proved to be highly important. The importance of microgravity as a reference parameter for research in the field of gravitational biology was clearly demonstrated.

STATOCYTE POLARITY AND STATOLITHS BEHAVIOR UNDER MICROGRAVITY CONDITIONS

Actomyosin counteracts gravity

Experiments investigating cress and lentil roots (39–42) demonstrated that in principle the structural polarity of statocytes is established and maintained under microgravity conditions, i.e., nucleus and ER membranes are located at the proximal and distal cell pole of statocytes, respectively. Calculations of the position of the nucleus showed that its location changed slightly. This indicates that even the position of the nucleus depends on the gravitational force (25, 43). Astonishingly, non-sedimented statoliths did not show random distribution, as might be expected, but showed remarkable shifts in direction of the nucleus, indicating active motion of the organelles (13, 40). In addition, it has been shown that statoliths in microgravity were grouped near the cell center, whereas they appeared more dispersed after treatment on the slowly rotating clinostat (44). Direct evidence for actively driven movement of statoliths has come from experiments on rockets when, after launch accelerations of some g, gravitational conditions changed immediately to microgravity. Under these experimental conditions, statoliths moved in the opposite direction to the originally acting gravity vector (Fig. 2) within a few minutes (16). These results were also observed during shuttle experiments (Fig. 3) (43, 45, 46). Corresponding behavior of statoliths from the Chara rhizoid was directly observed in orbit by telecommunication (16). Under cytochalasin D action this motion did not occur and statoliths remained in their launch position (47, for review see 48). Microgravity effects on actin isoforms have been observed with plant protoplasts. In sounding rocket experiments two of four actin isoforms nearly disappeared after 6 min of microgravity (49).

Threshold values indicate stimulus transformation in close vicinity of statoliths

Microgravity experiments offered, for the first time, possibilities to study important parameters like thresh-
old values under controlled conditions of sensor physiology. For plant material cultivated entirely under microgravity conditions, the minimum dose under continuous stimulation (presentation time $t_p$; for details see ref. 21) has been estimated to be 20–30 gs for cress roots (50), and by extrapolation from microgravity data to be 27 gs for lentil roots (45). In contrast, this threshold value doubled (50–60 gs) when material from the 1-g centrifuge in orbit was used (21, 50).

Because root elongation is similar both in microgravity and on the 1-g centrifuge (51), these results indicate that gravisensitivity of roots is larger when the organs develop in microgravity than when they are growing under 1-g conditions. Correlations of this threshold value with the position of statoliths show that statoliths moved very slightly in the direction of the stimulating gravity vector, generally less than 1 $\mu$m. Thus one must conclude that stimulus transformation occurs in close vicinity to the statoliths after short distance movements.

CONCLUSIONS

From both ground-based studies and microgravity experiments, it can be concluded that cytoskeletal proteins, especially actin and its associated proteins, play an important role in (1) development and maintenance of structural polarity in root cap statocytes; (2) the motion and sedimentation of statoliths; and probably (3) the transformation of the gravity stimulus into the first biochemical signal. So far, prominent actin microfilament bundles in statocytes have not been demonstrated but microgravity experiments indicate that actomyosin-driven motion of statoliths counteracts the sedimentation process caused by the gravitational force. These results are supported by immunolabeling of actin and myosin in the close neighborhood of statoliths. On the basis of this behavior of statoliths, it can be hypothesized that gravity-perceiving cells are forming the first biochemical signal. Stimulus transformation occurs in close vicinity to the statoliths after short distance movements.

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