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CELLULAR POLARITY AND INTERACTIONS IN PLANT GRAVIPERCEPTION

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Our long term goal is to understand the mechanisms of gravitropic sensing in higher and lower plants. This involves identifying the cells that sense gravity and determining the cellular mass that gravity acts upon to trigger sensing. We hope to learn which events occur during the transduction of a physical signal into a physiological signal that affects growth.

A second goal is to understand how cells have "used" gravity in orienting and organizing themselves. A related question is what mechanisms have evolved that prevent the stratification of cell components with respect to gravity?

Accomplishments

Gravity sensing away from the rootcap

We found that gravitropic roots of the aquatic angiosperm, *Limnobium* have sedimented amyloplasts in their elongation zone but not in their rootcap. These data extend our previous findings from roots of the more primitive plant, *Equisetum* that amyloplast sedimentation in roots can occur outside the cap. While this does not prove that the elongation zone is capable of sensing, it does provide structural data that warrant a reinvestigation of the question of whether sensing is *confined* to the cap in all roots.

We also found that in *Limnobium* roots, the nuclei as well as the amyloplasts sediment in the elongation zone. As far as I know, these cells in roots of *Limnobium* and *Equisetum* are the only cases reported where nuclei sediment at 1 *g*. Presumably this demonstrates that nuclei have enough mass to sediment in other systems as well, but do not do so, perhaps because of restraint by the cytoskeleton.

Gravity and Moss Protonemata

The tip cell of the protonema of the moss *Ceratodon* senses and responds to gravity by adjusting the direction of its growth. It is the only single-celled plant system, above the level of the algae, whose gravitropism has been studied in much detail.

In the last year of the grant, we attempted to understand how plastid sedimentation occurs in vertical cells and how this sedimentation is regulated. Sedimentation is clear in horizontal cells but previously we did saw no obvious sedimentation in vertical cells since plastids were distributed throughout the lenght of upright cells. However, by specifically comparing upright and inverted cells it became clear that amyloplast sedimentation does occur along the length of vertical protonemata.

It is clear that this cell is very different from cells in higher plants that contain amyloplast sedimentation since in the latter virtually all the amyloplasts in the cell sediment and they do so to the lowermost wall. In contrast, in the *Ceratodon* protonemata, only some amyloplasts fall and only in some locations. Furthermore, the extent of sedimentation appears to be tightly regulated since there are plastids distributed throughout the length of the cell.

These observations led us to ask what component was allowing yet restricting sedimentation. To determine whether the cytoskeleton restricts plastid sedimentation, the effects of amiprophos-methyl (APM) and cytochalasin D (CD) on plastid position were quantified. APM treatments of 30-60 minutes increased the plastid sedimentation that is normally seen along the length of untreated or control cells, and induced the sedimentation of plastids that do not sediment in untreated cells. Longer APM treatments often resulted in more dramatic plastid sedimentation, and in some cases almost all plastids sedimented to the lowermost point in the cell. In contrast, the microfilament inhibitor CD did not affect longitudinal plastid sedimentation compared to untreated cells, although it did disturb or eliminate plastid zonation in the tip. These data suggest that microtubules restrict the sedimentation of plastids along the length of the cell and that microtubules are load-bearing for all the plastids in the apical cell. This demonstrates the importance of the cytoskeleton in maintaining organelle position and cell organization against the force of gravity.

Another accomplishment concerned the study of other moss protonemata. Protonemata of the mosses *Ceratodon* and *Physcomitrella* are gravitropic and substantial amyloplast sedimentation occurs in *Ceratodon*. This study attempted to extend these findings by examining whether caulonemata of the moss *Funaria* also are gravitropic, and whether *Funaria* and *Physcomitrella* caulonemata contain amyloplasts that sediment. It was determined that *Funaria* caulonemata grow upwards in the dark, i.e. that they are negatively gravitropic. Amyloplast sedimentation occurred in *Funaria* tip cells regardless of whether the cells were horizontal, upright or inverted. However, sedimentation was incomplete since amyloplasts did not sediment entirely to the bottom of the cell in any orientation. Application of the microtubule inhibitor, amiprophos-methyl (APM) enhanced amyloplast sedimentation, suggesting that microtubules are load-bearing for plastids in these cells. In untreated tip cells of *Physcomitrella*, some amyloplast sedimentation was found in the subapical region. Comparison of *Physcomitrella*, *Funaria* and *Ceratodon* protonemata indicates that the amount of gravitropic curvature correlates more with the extent of plastid sedimentation than with the rate of tip extension. These results suggest that gravitropism may be relatively common in moss protonemata and reinforce the idea that amyloplast mass functions in gravitropic sensing.

GRAVITY-RELATED PUBLICATIONS STEMMING FROM GRANT

Ridge, R & FD Sack 1992 Cortical and cap sedimentation in gravitropic *Equisetum* roots. Amer. J. Bot. 79: 328-334

Young, JC & FD Sack 1992 Time-lapse analysis of gravitropism in *Ceratodon* protonemata. Amer J Bot. 79: 1348-58

Sack, F 1992 Gravitropism in protonemata of the moss *Ceratodon*. Bulletin of the Torrey Botanical Club 119 (2): vi

Sack, F 1992 The cell biology of plant gravity sensing. Abstracts of World Space Congress, COSPAR meeting, Washington DC, August

Walker, LM & FD Sack 1992 Ultrastructural analysis of dark grown protonemata of the moss *Ceratodon*. Amer. Soc. Gravitational Space Biol. Bull. 6(1): 38

Schwuchow, J & FD Sack 1992 Effects of inversion on plastid position and gravitropism in *Ceratodon* protonemata. Amer. Soc. Gravitational Space Biol. Bull. 6(1): 75

Schwuchow, J & FD Sack 1992 The influence of cytoskeletal inhibitors on plastid position in inverted and vertical *Ceratodon* protonemata. Amer. Soc. Gravitational Space Biol. Bull. 6(1): 96

Sack, F 1993 Gravitropism in protonemata of the moss *Ceratodon*. Bulletin of the Torrey Botanical Club, 25: 36-44 (review)

Schwuchow, J & F Sack 1993 Effects of inversion on plastid position and gravitropism in

Ceratodon protonemata. Can J Bot 71: 1243-8

Sack, FD, D Kim & B Stein 1993 Organelle sedimentation in gravitropic roots of *Limnobium* is restricted to the elongation zone. Amer Soc Gravitational & Space Biol Bull 7: 91

Sack, FD & J Schwuchow 1993 Microtubules limit plastid sedimentation in protonemata of the moss *Ceratodon purpureus*. #3020 in abstracts of XV International Botanical Congress, Yokohama

Sack, FD, D Kim & B Stein 1994 Organelle sedimentation in gravitropic roots of *Limnobium* is restricted to the elongation zone. Annals Bot, in press

Schwuchow, JM & FD Sack. Microtubules restrict plastid sedimentation in protonemata of the moss *Ceratodon*. Submitted for publication.

Schwuchow JM, D Kim & FD Sack. Protonemal gravitropism and amyloplast sedimentation in the moss *Funaria hygrometrica*. Submitted for publication.