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Conservation of the Plastid Sedimentation Zone in All Moss Genera with Known Gravitropic Protonemata

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ABSTRACT

Moss protonemata from several species are known to be gravitropic. The characterization of additional gravitropic species would be valuable to identify conserved traits that may relate to the mechanism of gravitropism. In this study, four new species were found to have gravitropic protonemata, *Fissidens adianthoides*, *Fissidens cristatus*, *Physcomitrium pyriforme*, and *Barbula unguiculata*. Comparison of upright and inverted apical cells of *P. pyriforme* and *Fissidens* species showed clear axial sedimentation. This sedimentation is highly regulated and not solely dependent on amyloplast size. Additionally, the protonemal tip cells of these species contained broad subapical zones that displayed lateral amyloplast sedimentation. The conservation of a zone of lateral sedimentation in a total of nine gravitropic moss species from five different orders supports the idea that this sedimentation serves a specialized and conserved function in gravitropism, probably in gravity sensing.

Key words: *Barbula*; *Fissidens*; Gravitropism; Moss; *Physcomitrium*; Plastid; Protonema; Sedimentation

INTRODUCTION

Gravitropic organs of higher plants and tip-growing moss cells are fundamentally different in cellular organization but are similar in that both contain amyloplasts that sediment. In gravitropic multicellular organs, sedimentation is separated from the zone of curvature by many cells and is confined to specialized cell types such as the starch sheath in stems and columella cells in roots. In these cells, essentially all amyloplasts sediment to the bottom of the cell (Sack 1991, 1997). In contrast, in the apical cells of moss protonemata that are gravitropic, sedimentation and curvature take place within a single cell, only a fraction of amyloplasts sediment, and they do not sediment to the bottom of the cell (Schwuchow and others 1995; Walker and Sack 1990). Numerous data support the hypothesis that the mass of amyloplasts that sediment somehow trigger gravitropic sensing in higher plants (reviewed in Kiss 2000; Sack 1997; Tasaka 2001) as well as in moss protonemata (Kuznetsov and others 1999; Sack and others 1998; Sack and others 2001).

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Protonemata of the mosses *Ceratodon, Funaria, Physcomitrella, Pottia,* and *Pohlia* are known to be negatively gravitropic in the dark and to have a specialized zone of amyloplast sedimentation (Chaban and others 1998; Cove and others 1978; Demkiv and others 1999; Schwuchow and others 1995; Walker and Sack 1990). In this zone, which is located behind the apical dome, amyloplasts sediment towards the lower side wall in horizontal cells (lateral sedimentation). In *Ceratodon* and *Pottia,* amyloplasts are also known to sediment along the length of the cell (axial sedimentation), as shown by comparing upright and inverted cells (Chaban and others 1998; Kern and others 2001; Schwuchow and Sack 1993). However, amyloplasts never fall all the way to the bottom of the cell unless microtubules are depolymerized (Schwuchow and Sack 1994). Thus, in all orientations plastid sedimentation is highly regulated.

Despite this regulation overall, there are variations in the extent of sedimentation and in the number and size of amyloplasts between different moss genera. Also, the best studied taxon, *Ceratodon,* has non-sedimenting plastids in the cell apex, whereas in other genera, plastids are virtually absent from the apical dome (Schwuchow and others 1995). One way to determine which traits are critical for gravitropism is to identity additional genera whose apical cells grow opposite to the gravity vector. We found four new species with gravitropic protonemata and report that all display a conservation of the lateral zone of amyloplast sedimentation.

MATERIALS AND METHODS

Plant Material and Culture Conditions

Aseptically propagated moss stock cultures were a generous gift of Malcolm Sargent, University of Illinois (Sargent 1988). This collection included a total of 19 taxa from 12 genera, that is, *Anacamptodon splachnoides, Atrichum angustatum, Barbula unguiculata, Dicranum scoparium, Fissidens adianthoides, Fissidens cristatus, Fissidens osmundoides, Leucobryum glaucum, Mnium cuspidatum, Physcomitrium pyriforme, Polytrichum commune, Polytrichum formosum, Polytrichum juniperinum, Pylaisiella selwynii, Sphagnum centrale, Sphagnum compactum, and Tortella tortuosa.*

Stock cultures were maintained in a growth chamber at 21–22°C on a 16 h photoperiod of 80–90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light from General Electric 17 W fluorescent lamps. Gametophores from stock culture tubes were fragmented by a blender in sterile distilled water and transferred with a pipette onto fresh medium in sterile Petri dishes (Falcon, 50 mm diameter). The medium used was slightly modified from that used by Lamparter and others (1996) and contained 40 μM $MgSO_4 \cdot 7H_2O$, 1 mM KH_2PO_4, 1 mM KNO_3, 10 μM $C_6H_5FeO_7$, 0.1 mM $CaCl_2 \cdot 2H_2O$, 11 mM glucose, trace elements (0.22 μM $CuSO_4 \cdot 5H_2O$ 10 μM H_3BO_3, 0.23 μM $CoCl_2 \cdot 6H_2O$, 0.1 μM $Na_2MoO_4 \cdot 2H_2O$, 0.2 μM $ZnSO_4 \cdot 7H_2O$, 2 μM $MnCl_2 \cdot 4H_2O$, 0.17 μM KI), and 1.2% (w/v) agar (Sigma Chemical Company, St. Louis, MO, USA). The pH was adjusted to 5.8 with KOH. We chose this medium for all experiments to facilitate a direct comparison of the extent of protonemal growth, curvature, and plastid sedimentation among different genera. A comparable medium was used previously to study protonemata of *Ceratodon, Funaria, Physcomitrella,* and *Pottia* (Chaban and others 1998; Kern and Sack 1999; Schwuchow and others 1995).

Protonemata can develop from spores that germinate, or from moss shoots (gametophores) that are subcultured or wounded (secondary protonemata; Chaban and others 1998). Because the stock cultures had been repeatedly subcultured, and because the cultures were homogenized using a blender, protonemata did not arise from spores or from intact gametophores. Instead, protonemata developed from fragments of gametophores, preexisting protonemata, and perhaps from rhizoids.

Pieces of generated protonemal cultures were aseptically transferred using forceps and sown in a line onto nutrient medium that was overlaid with cellophane (Figure 1; Kern and Sack 1999). For *Fissidens* and *Physcomitrium*, mostly protonemata were sown. For *Barbula*, both protonemata and gametophore fragments were sown. The dishes were sealed with parafilm and kept in darkness with the agar surface oriented vertically.

For the time course of gravitropic curvature as well as other experiments, the dishes were kept upright in darkness for 12–18 days (*Physcomitrium, Fissidens*) or 3–4 weeks (*Barbula*) and then rotated (see below).

Experimental System, Microscopy, and Data Analysis

Culture dishes were rotated 90° (horizontal) for 1, 2, 3, 4, 6, 8, and 24 h or 180° (inversion) for 4 h in darkness. After gravistimulation, protonemata were fixed in position for 1 h in 1% [w/v] paraformaldehyde, 2% [v/v] glutaraldehyde, 50 mM PIPES (1,4-piperazinediethanesulfonic acid) buffer and 5 mM $CaCl_2$, at pH 7.

Chemically fixed protonemata were stained for starch with IK_2I and photographed using an Olympus AX70 compound microscope and an Optronics digital camera (MagnaFire S60800). Plastid distribution was determined from the monitor, and lateral sedimentation was analyzed by assessing qualitatively the extent of asymmetrical distribution of plastids with respect to the axial cell midline. Tip angle was measured from the monitor using customized software, with 0° corresponding to the horizontal and 90° to the new vertical orientation. Growth rates were determined by measuring the length of the curved cell wall in 10 horizontally stimulated cells for each gravitropic species. Plastid sizes were measured by digitizing plastid diameters from 10 micrographs of fixed and IK_2I stained cells using NIH image software (http://rsb.info.nih.gov/nih-image) and a customized software macro.

RESULTS

Culture Growth

Nine of the genera tested (see Materials and Methods) did not form many protonemata in the dark on the medium used. Three of the 12 genera *Physcomitrium*, *Fissidens,* and *Barbula* displayed reasonable protonemal growth in the dark, meaning that these cultures had at least several long protonemal filaments. Cultures from the above three genera contained protonemata that were negatively gravitropic (Figure 1). The extent and timing of protonemal formation varied among these three genera. The average number of long protonemata per culture that regenerated in the dark was only 3.8 ± 0.8 (±SE) for *Barbula unguiculata*, compared with 25.8 ± 1.5 for *Physcomitrium pyriforme* and 34.2 ± 2.9 for *Fissidens adianthoides*. However, all of the above species produced many fewer protonemata than *Ceratodon* (>200 filaments per culture, see Sack and others 1998).

Time Course of Curvature

Figure 1 shows cultures containing protonemata that grew up in the dark along the surface of the

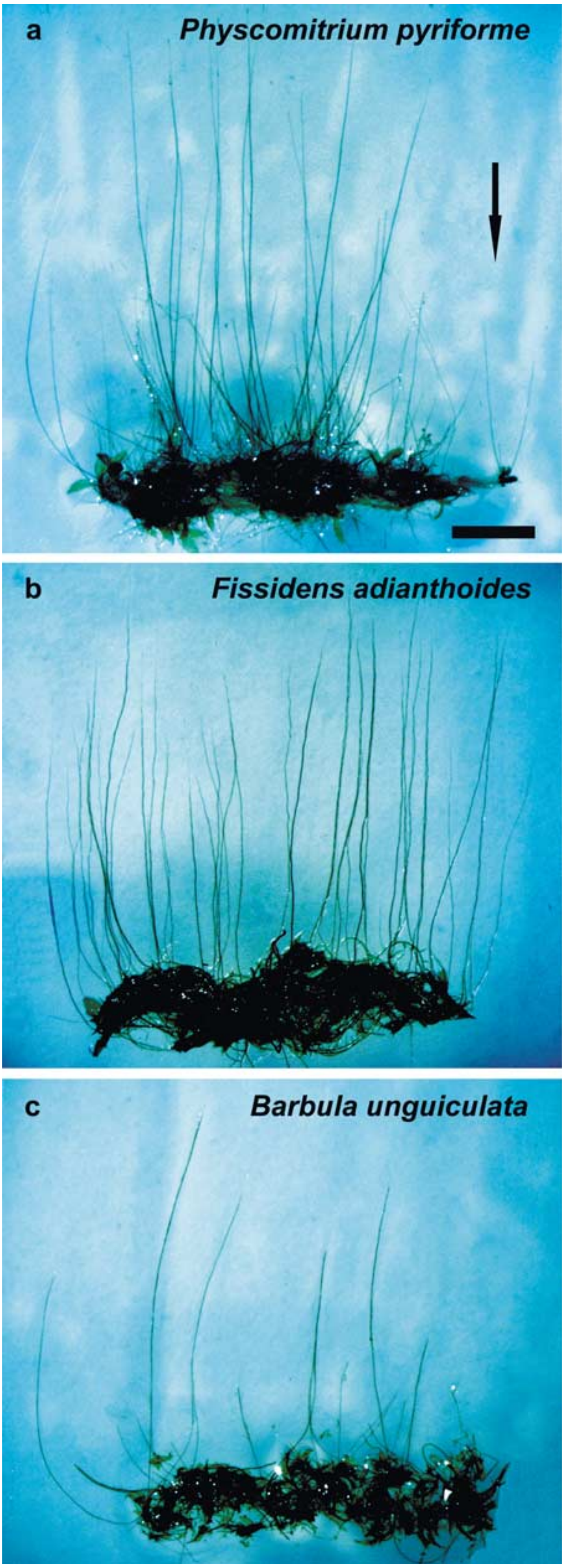

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Figure 1. Cultures of **(a)** *Physcomitrium pyriforme* (12 d dark growth), **(b)** *Fissidens adianthoides* (14 d in dark) and **(c)** *Barbula unguiculata* (26 d in dark). Dishes were turned on edge so that the agar surface was vertical. The dark line of moss at the bottom of each figure developed from sowing fragments in a line. Note upright growth of protonemata in all cultures, and the higher density and growth rate of protonemata in *P. pyriforme* and *F. adianthoides.* Arrow: direction of gravity vector. Bar in **(a)** is 2 mm for all three cultures.

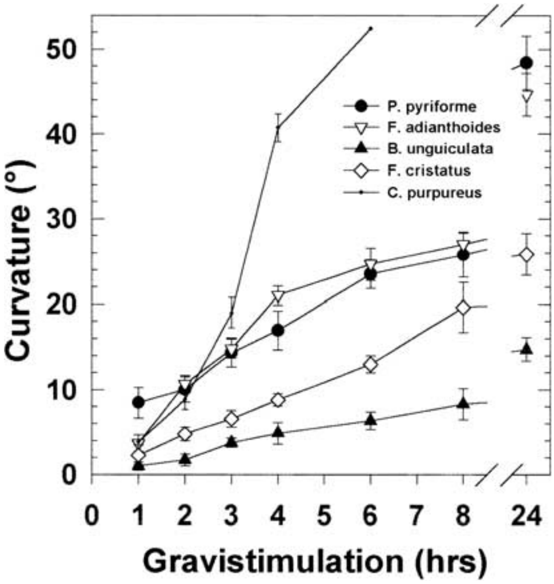

Figure 2. Time course of upward gravitropic curvature of protonemata from *Physcomitrium pyriforme, Fissidens adianthoides, Fissidens cristatus,* and *Barbula unguiculata.* Data from *Ceratodon purpureus* are included for comparison (see text). Each time point represents the mean curvature of 20–59 fixed protonemata (3–16 protonemata per time point for *B. unguiculata* due to sparse growth). Vertical bars represent standard errors.

agar. To determine the rate and duration of curvature, cultures with upright protonemata were turned to the horizontal and then chemically fixed after different periods of gravistimulation. Curvature of *Physcomitrium*, *Fissidens adianthoides*, and *F. cristatus* could be detected after 1–2 h of gravistimulation, and curvature of *Barbula* was seen after 2–3 h (Figure 2). Mean curvature (±SE) after 24 h was, in descending order, 48.4 ± 3.2° for *Physcomitrium*, 44.6 ± 2.5° for *Fissidens adianthoides*, 25.9 ± 2.4° for *F. cristatus*, and 14.7 ± 1.4° for *Barbula*.

Gravitropic curvature correlated with the rate of tip growth (extension). The two species with the highest rate of curvature had the fastest growth (*P. pyriforme* 38.6 ± 3.3 μm/h, *F. adianthoides* 29.8 ± 1.6 μm/h), whereas the slower curving *F. cristatus* and *B. unguiculata* extended more slowly (21.2 ± 2.3 and 11.5 ± 2.6 μm/h, respectively).

The time course of the initial bending phase in *Ceratodon purpureus* was included for comparison (see Kern and Sack 1999; Young and Sack 1992). Curvature of *Ceratodon* protonemata was comparable to that of *P. pyriforme* and *F. adianthoides* during the initial phase (<3 h) but later (≥4 h) *Ceratodon* curved much faster and more than all other species.

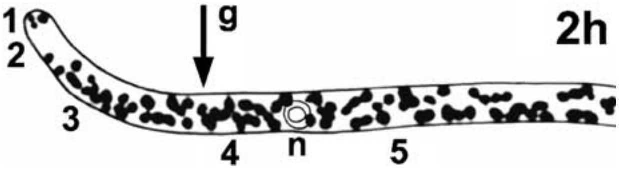

Figure 3. Tracing of a tip cell of *Ceratodon purpureus* that was gravistimulated for 2 h showing zones of plastid distribution: (1) non-sedimenting tip plastids, (2) plastid-free zone, (3) sedimentation zone, (4) zone with no or little sedimentation, (5) zone containing non-sedimenting plastids and one or more vacuoles. Arrow: direction of the gravity vector, n: nucleus.

Cytology of Horizontally Stimulated Cells

The plastid numbering system described for *Ceratodon* (Sack and others 1998; Figure 3) is used as a reference for genera in the current study. Horizontal apical cells of *Ceratodon* display five plastid zones: (1) a group of non-sedimenting plastids in the apex, (2) a plastid-free zone, (3) a zone where amyloplasts sediment towards the lateral wall, (4) a region before the nucleus with little or no sedimentation, and (5) a zone of non-sedimenting plastids located between the nucleus and the basal cell wall.

All four species that were negatively gravitropic had protonemata with characteristics of caulonemata. These included oblique cell walls, some spindle shaped amyloplasts, and a pronounced plastid polarity and zonation (Duckett and others 1998; Sack and others 1998; Tewinkel and Volkmann 1987). This zonation was mostly similar to *Ceratodon* (Figures 3 and 4). However, zones similar in plastid behavior but different in location compared to *Ceratodon* are indicated with an asterisk ((*) in Fig. 4a; see below). Staining with IK_2I revealed that all mature plastids in dark grown tip cells were amyloplasts, that is, they contained abundant starch.

Upon horizontal gravistimulation, amyloplasts sedimented towards the lower cell wall in a specific zone behind the tip (arrowheads in Figure 4). Sedimentation could be detected as early as 1 h in *Physcomitrium* and *Fissidens*, and after about 2 h in *Barbula*.

Lateral sedimentation was clearly visible in all species, but it varied and in our experiments amyloplasts did not sediment to the bottom of the cell. In *Physcomitrium* and *Barbula*, sedimentation was subtler and the sedimentation zone was usually shorter than in *F. adianthoides* and *F. cristatus*.

All tip cells had a plastid-free zone (zone 2 in Figure 4), a zone of distinct amyloplast sedimentation (zone 3), and one or more zones of non-sedi-

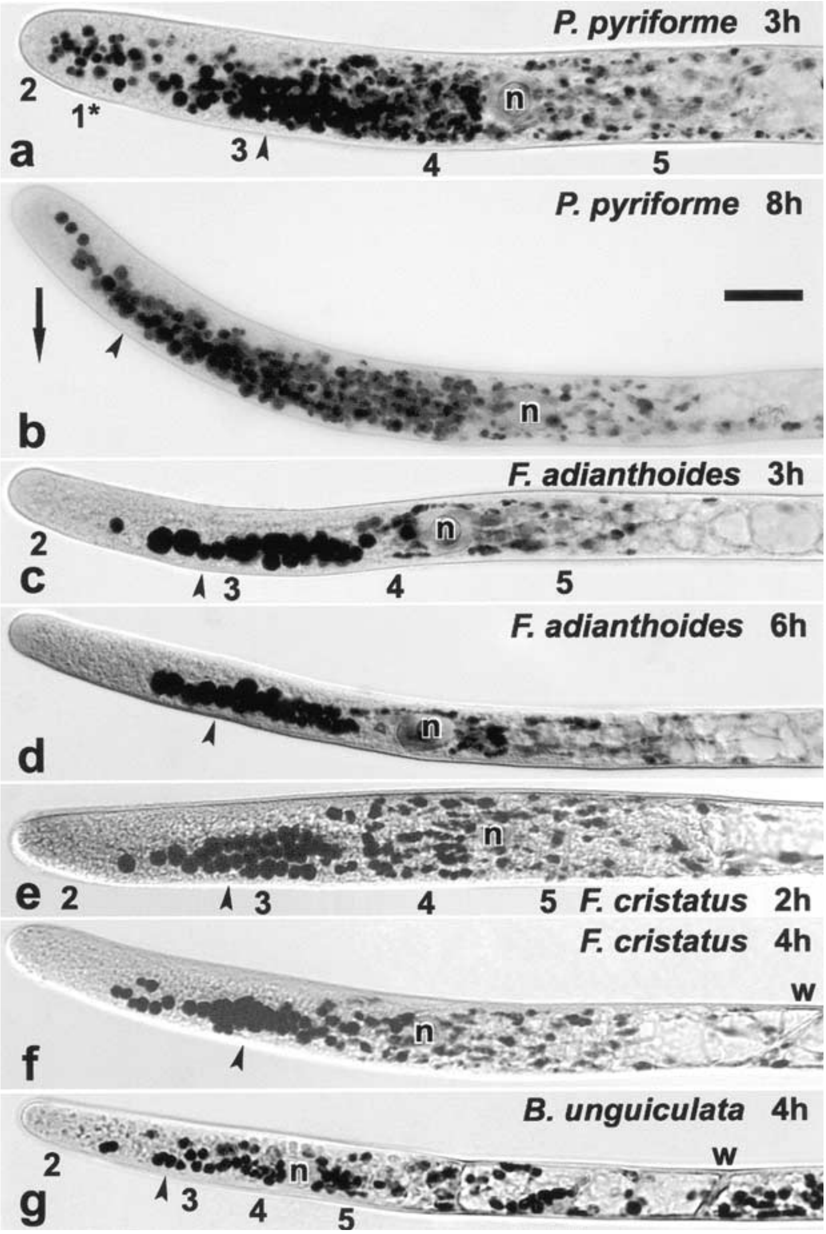

Figure 4. Upward gravitropism and amyloplast sedimentation in horizontally stimulated protonemal apical cells. **(a, b)** *Physcomitrium pyriforme*, **(c, d)** *Fissidens adianthoides*. **(e, f)** *F. cristatus*, and **(g)** *Barbula unguiculata*. The number of hours of horizontal gravistimulation are shown for each micrograph. Arrowheads denote location of lateral plastid sedimentation. Arrow in **(b)** shows direction of gravity for entire figure. Plastid zones are described in the text. n: nucleus. ''w'' marks the basal cell wall, where visible. Bar in **(b)** represents 20 μm for all micrographs.

menting plastids (zone 1*, zones 4 and/or 5). However, the typical plastid zonation and the length of the plastid-free zone differed significantly among the three genera.

Physcomitrium (Figures 4a, b) had a relatively short (10–15 μm long) plastid-free zone in the apical dome (Figure 4a - zone 2). The plastid-free zone in *Fissidens* and *Barbula* was about 2–3 times longer (Figures 4c–g). *Physcomitrium* differed from *Barbula* and *Fissidens* in having a zone of non-sedimenting plastids located very close to the tip (Figure 4a - zone 1*). Unlike *Ceratodon*, which has non-sedimenting plastids right in the apical dome, in *Physcomitrium*, this zone is sub-apical and located between a plastid-free zone and the more basal lateral sedimentation zone (Figure 3).

All four species had a zone with less lateral amyloplast sedimentation (zone 4), a zone that extended to the nucleus. The region basal to the nucleus (zone 5) contained vacuoles and non-sedimenting plastids; these plastids were usually smaller than those located on the apical side of the nucleus and were dumbbell or spindle-shaped, in contrast to the more spherical or ellipsoid plastids between tip and nucleus.

The extent of gravitropic curvature was related to the mean diameter of plastids that were located between the cell tip and the nucleus. For example,

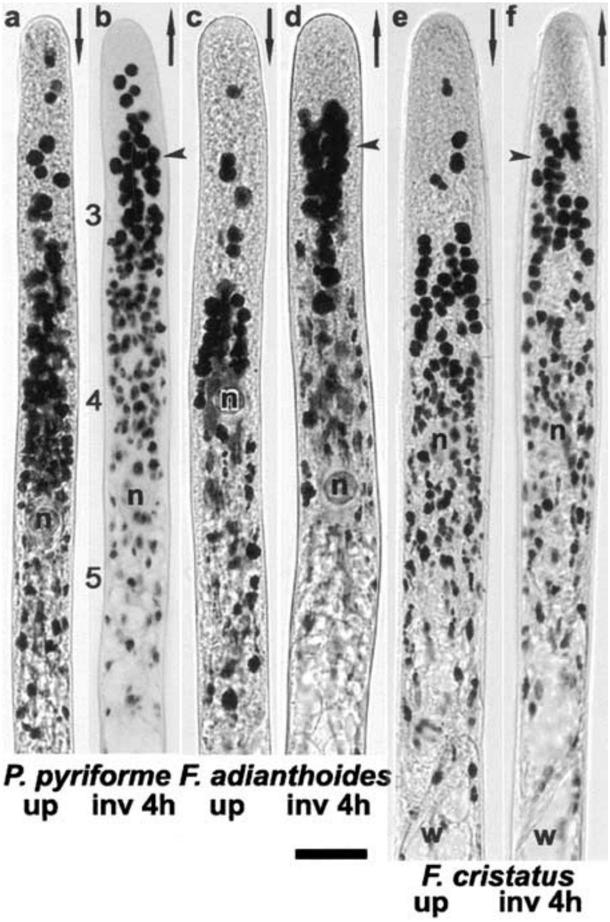

Figure 5. Comparison of plastid position in upright **(a, c, e)** and 4 h inverted **(b, d, f)** protonemal tip cells of **(a, b)** *Physcomitrium pyriforme,* **(c, d)** *Fissidens adianthoides,* and **(e, f)** *Fissidens cristatus.* Note that the micrographs of the inverted cells **b, d,** and **f** have been rotated to facilitate comparison with upright cells. Stained for starch with IK$_2$I. Arrowheads show axial plastid sedimentation towards the tip. Note that plastids do not sediment all the way into the tip in inverted cells. Arrows: direction of the gravity vector. "n" nucleus. "w" cell wall. Bar 20 μm.

the fastest curving protonemata, *Physcomitrium* and *F. adianthoides,* had the largest plastids (mean diameters of 3.33 ± 0.04 (SE) μm and 4.17 ± 0.09 μm, respectively) whereas slower curving cells of *F. cristatus* and *Barbula* had significantly smaller plastids (2.97 ± 0.04 and 2.56 ± 0.05 μm, respectively). Although the rate of curvature was related to mean plastid size, it did not correlate with mean plastid number (data not shown).

Inversion

Upright and inverted cells of all four species displayed highly regulated amyloplast sedimentation along the length of the cell. Some amyloplasts did not fall, and those that did sediment, did not fall all the way to the bottom of the cell. Thus, the existence of axial plastid sedimentation is best displayed when upright and inverted cells are compared side by side (Figure 5).

In inverted cells, plastids sedimented towards the apex, but did not fall all the way into the apical dome (Figure 5). The length of the plastid-free zone in the tip frequently decreased after inversion, but this zone never disappeared, and the typical zonation was always maintained. Amyloplasts in inverted cells moved within zones, but they also moved through zones. The difference in plastid distribution in upright and inverted cells is most obvious in the lateral sedimentation zone, that is, in the zone where amyloplasts fall to the side wall in horizontal cells (compare Figures 4 and 5). This is because many plastids accumulated at the apical end of the sedimentation zone in inverted cells, whereas this region often had fewer plastids in upright cells.

In *Fissidens,* inversion resulted in the sedimentation of amyloplasts from zone 4 (just apical to nucleus) into zone 3 (lateral sedimentation zone), as well as amyloplasts within zone 3 sedimenting towards the bottom of that zone (Figure 5, compare upright cells c, e with inverted d, f). In inverted cells, often many large spherical plastids from zone 3 sedimented towards the tip and frequently accumulated near the plastid-free zone (arrowheads in Figures 5d and f). Some of the dumbbell or spindle-shaped plastids from zones 4 and 5 (basal to nucleus) also sedimented towards the tip but did not fall into the region containing the larger spherical plastids.

Plastids were not solely distributed according to their size. In some cases, axial sedimentation within zone 3 correlated with plastid size, but this was not necessarily true in zone 4. However, in upright cells, often the largest plastids were also closer to the tip and the smaller plastids were near the nucleus (Figures 5a and e). This was particularly true in *F. adianthoides,* the species with the largest plastids and highest gradient of plastid sizes (ranging from 1 to 11 μm in diameter). This same pattern could also be detected in many upright protonemata of *Ceratodon* (data not shown), suggesting that amyloplast sedimentation is regulated primarily within zones and that plastid size may play only a secondary role.

DISCUSSION

This work has identified four new moss species that are negatively gravitropic, that have amyloplasts

Table 1. Moss Species Known to have Gravitropic Protonemata

Order/Family[a]	Binomial	Amyloplast Sedimentation (+++ Greatest)	Growth Rate [μm/h ± SE]	Time to Reach 40° Curvature [h]
Pottiales/ Pottiaceae	*Barbula unguiculata*	+	9.3 ± 1.8	65
	Pottia intermedia	++[b]	25–30[b]	18[b]
Dicranales/ Ditrichaceae	*Ceratodon purpureus*	+++[c]	25–30[d]	4[c]
Fissidentales/ Fissidentaceae	*Fissidens adianthoides*	+++	29.8 ± 1.6	22
	Fissidens cristatus	++	21.2 ± 2.3	37
Funariales/ Funariaceae	*Funaria hygrometrica*	++[e]	45–48[e,f]	24[e]
	Physcomitrella patens	+[e]	20–30[g]	30–36[g]
	Physcomitrium pyriforme	++	38.6 ± 3.3	20
Bryales/ Bryaccae	*Pohlia nutans*	++[h]	9.1 ± 0.3[h]	24[h]

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[a] *Crum and Anderson 1981*
[b] *Chaban and others 1998*
[c] *Walker and Sack 1990*
[d] *Young and Sack 1992*
[e] *Schwuchow and others 1995*
[f] *Schmiedel and Schnepf 1980*
[g] *Jenkins and others 1986*
[h] *Demkiv and others 1999*

that sediment to the side wall in a specific zone in horizontal cells, and that display highly regulated, partial sedimentation along the length of the cell. The conservation of specific and complex patterns of amyloplast sedimentation in all moss species known to have gravitropic protonemata suggests strongly that amyloplast mass functions in gravity sensing in this unique single-celled system.

Detection of Gravitropism

Protonemal gravitropism probably guides filaments from buried spores to grow upward until they reach the light. The percentage of moss species that display this gravitropism is unknown. The detection of protonomal gravitropism depends on how well protonemata grow in the dark in sterile culture. This is because light eliminates the gravitropic response and because exogenous sugars are necessary to support cell growth (Kern and Sack 1999).

Four of the 19 moss species studied here produced readily detectible protonemata in the dark that were gravitropic, that is, *Fissidens adianthoides, F. cristatus, Physcomitrium pyriforme,* and *Barbula unguiculata.* None of these produced as dense a mat of protonemata as a strain of *Ceratodon* that has been used for studies of gravitropism and phototropism (Hartmann and others 1983; Kern and Sack 1999). This is partly because protonemata of *Physcomitrium, Fissidens,* and *Barbula* develop quickly into shoots whereas this strain of *Ceratodon* rarely does.

Most of the other mosses examined here did not produce detectable protonemata in the dark. Some species produced a small number of protonemata that did not appear to be gravitropic. Limited protonemal growth could result from an inability to initiate protonemata from shoots, from the lack of extension of protonemal primordia, from a failure to use exogenous sugar to support growth and development in the dark, and from a lack of optimization of growth media for individual species. In any case, this study adds three new genera of mosses, *Physcomitrium, Fissidens,* and *Barbula* to the five known to have gravitropic protonemata, that is *Ceratodon, Funaria, Physcomitrella, Pottia,* and *Pohlia* (Table 1) (Demkiv and others 1999; Sack and others 1998).

Conservation of the Lateral Sedimentation Zone

Comparison of horizontal cells of the four species in this study (Figure 4) with previously described gravitropic moss protonemata (Figure 6), demonstrates that a broad lateral sedimentation zone is conserved in all known gravitropic protonemata. In

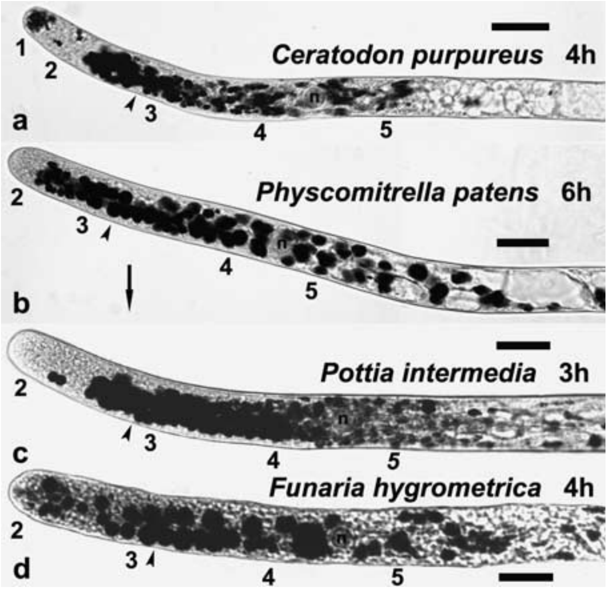

Figure 6. Micrographs of horizontal protonemal tip cells of **(a)** *Ceratodon purpureus*, **(b)** *Physcomitrella patens*, **(c)** *Pottia intermedia*, and **(d)** *Funaria hygrometrica*. Numbers indicate hours of horizontal gravistimulation. Arrowheads show location of lateral plastid sedimentation. Arrow: direction of the gravity vector. n-nucleus. Bars 20 µm. Figures of *Pottia* and *Funaria* are reproduced from Chaban and others (1998) and from Schwuchow and others (1995).

all cases, amyloplast sedimentation takes place towards the side wall in horizontally stimulated cells. This zone starts between 20 and 40 µm from the cell apex and extends for 40 to 80 µm in a basal direction (towards the nucleus).

Many lines of evidence support the hypothesis that amyloplasts that sediment function in gravitropic sensing in moss protonemata (Sack and others 2001). For example, manipulation of amyloplast position using a high gradient magnetic field mimics gravitropism in both wild type and in the *wrong way response* mutant of *Ceratodon* (Kuznetsov and others 1999). Also, when protonemata are centrifuged basipetally, gravitropism recovers after amyloplasts migrate back and sediment in this zone (Walker and Sack 1991).

The conservation of the sedimentation zone in nine gravitropic moss species from five different orders (Table 1) supports the idea that this sedimentation serves a specialized and conserved function in gravitropism—most likely in gravitropic sensing (Sack and others 1998). This conservation also supports the wider hypothesis that amyloplast mass triggers gravitropic sensing in the plant kingdom (Kiss 2000; Sack 1997; Tasaka 2001).

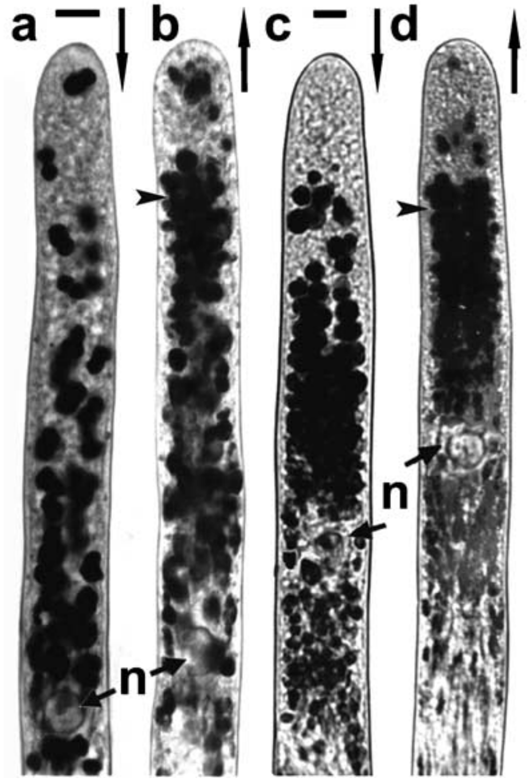

Figure 7. Plastid position in upright **(a, c)** and 1 h inverted **(b, d)** *Ceratodon* **(a, b)** and *Pottia* **(c, d)** protonemal tip cells. Arrowheads indicate axial plastid sedimentation towards the apex in inverted cells. Arrows show direction of the gravity vector. n-nucleus. Figures are reproduced from Schwuchow and Sack (1993) and from Chaban and others (1998). Bars 10 µm.

Other Zones and Variations

Other aspects of plastid zonation are either conserved or variant in gravitropic protonemata, depending upon the taxon. All nine genera have a plastid-free zone that is located somewhere between the tip of the apical cell and the sedimentation zone. All also have one or more zones of plastids that do not sediment significantly to the lateral wall in horizontal cells. In most cases, the zones of non-sedimenting plastids are basal to the sedimentation zone. *Physcomitrium* and *Ceratodon* are unique in that they have a zone of non-sedimenting plastids close to the tip (compare Figures 4 and 6). In *Ceratodon*, this zone is right in the apical dome and is followed by the plastid-free zone. In *Physcomitrium*, the reverse is true, that is, the tip lacks plastids and is followed by a zone of non-sedimenting plastids which at its base merges with the sedimentation zone.

In *Ceratodon*, the plastid-free zone contains many Golgi stacks which probably supply exocytic vesicles that contribute to tip growth (Sack and others 1998). Thus, we hypothesize that the plastid-free zone is conserved to allow for a concentration of Golgi stacks. *Ceratodon* has the only known gravitropic protonemata with a cluster of plastids on the apical side of the Golgi/plastid-free zone. Although the apical cluster would be expected to partially block exocytosis, *Ceratodon* is the most vigorously gravitropic species known.

We also compared the extent of sedimentation, amyloplast number, and size in different species with gravitropic protonemata (Table 1). The extent of sedimentation varies across species and is the most pronounced in protonemata of *Ceratodon* and *F. adianthoides* (Table 1). In other cases, sedimentation in horizontal cells is only detected by a thin plastid-free strip of cytoplasm near the upper wall (Figure 7: *Physcomitrella*) or by a few sedimented amyloplasts in a narrow zone (Figure 4: *Barbula*).

The extent of gravitropic curvature in the four species studied here correlated with mean plastid size, but not plastid number, between the tip and nucleus. A comparison of all gravitropic species (Table 1) shows that the rate of gravitropic curvature possibly correlates more with the extent of amyloplast sedimentation than with the rates of protonemal tip growth.

Complex Regulation of Sedimentation Along the Cell Length

Amyloplasts sediment along the length of vertical cells as well as to the side wall in horizontal cells. This has been most intensively studied in *Ceratodon,* but has also been documented for *Pottia* (Figure 7) (Chaban and others 1998; Kern and others 2001; Schwuchow and Sack 1993). Here we show that sedimentation along the cell length also occurs in *Fissidens* and in *Physcomitrium* (*Barbula* was not tested); thus axial sedimentation is a general phenomenon.

Gravitropic moss protonemata differ fundamentally from cells in tissues that show amyloplast sedimentation. In the latter, all the amyloplasts fall on top of each other at the bottom of the cell (Sack 1991). In moss apical cells, amyloplasts remain distributed along the length of the cell, but some amyloplasts sediment part way. This is why sedimentation along the cell length is best detected by comparing upright and inverted protonemata.

Sedimentation appears to be prohibited from certain zones such as the apical dome. In inverted cells of all five taxa that have been investigated, amyloplasts fall as far or further than the apical end of the lateral sedimentation zone, but not into the apical dome. Depolymerization of microtubules and microfilaments overcomes this prohibition and allows amyloplasts to fall into the very tip in inverted cells (Schwuchow and Sack 1994). However, cytoskeletal inhibitors also disrupt zone integrity and thus the cytoskeleton may act only indirectly in preventing sedimentation into the apex of inverted cells.

Microtubules do appear to be load bearing for amyloplasts that sediment along the length of the cell in *Ceratodon* (Schwuchow and Sack 1994). Depolymerization of microtubules, but not microfilaments, significantly enhances amyloplast sedimentation, especially of amyloplasts located between the nucleus and the apical end of the sedimentation zone.

These data show that amyloplast sedimentation along the length of the cell is regulated in a complex pattern. Microtubules appear to restrict sedimentation even though some sedimentation is allowed. Moreover, when *Ceratodon* protonemata are grown in microgravity or on a clinostat, amyloplasts become clustered in the ''sedimentation'' zone and superficially resemble inverted cells (Kern and others 2001). This suggests that endogenous forces normally act on amyloplasts and balance the effect of gravity in cells grown on earth. Together these data indicate that specific populations of amyloplasts sediment along the cell length and cluster subapically in microgravity.

Analysis of the size classes of amyloplasts that sediment along the cell length in *Physcomitrium pyriforme* and in *Fissidens adianthoides* may provide clues about the differential regulation of sedimentation. In both species, the largest amyloplasts sediment towards the tip upon inversion, whereas smaller amyloplasts remain nearer to the nucleus. In contrast, in *Ceratodon* and in *Pottia,* the size difference between sedimenting and non-sedimenting amyloplasts is not as obvious after inversion (Figure 7).

Gravitropic moss protonemata are in some ways more complex than the cells that may sense gravity in roots and stems. These multitasking moss cells sense gravity, grow differentially at their tip when reoriented, replicate plastids and other organelles, and undergo cell divisions that produce basal daughter cells that in turn form buds and shoots. They contain several different zones with specialized functions, many of which are unknown.

Gravitropic moss protonemata have unique patterns of plastid sedimentation. Plastid sedimentation takes place only in a few zones and plastids do not sediment all the way to the lower plasma membrane. Other plastids in other zones do not sediment. Even though plastid mass appears to play a role in sedimentation, especially within a single zone, the extent of sedimentation in tip cells is highly regulated and much more complex than in a mechanical system, where the speed and extent of sedimentation would be solely determined by particle mass. For example, in some upright cells, some large amyloplasts sediment towards the nucleus, while others remain at the apical end of the sedimentation zone; however, the latter are still capable of sedimenting when cells are turned to the horizontal. The conservation of such complex patterns of amyloplast sedimentation in different mosses suggests that these patterns are specializations for gravitropism, including for sensing.

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