

# Chapter 5

## Gravitropism in Fungi, Mosses and Ferns



Donat-Peter Häder

**Abstract** Motile pseudoplasmodia of cellular and acellular slime molds show gravitaxis while unicellular *Dictyostelium* amoebae do not. Fungi display gravitropism of their fruiting bodies which is thought to facilitate spore dispersal. Caulonemata and sporophytes of liverworts and mosses show negative gravitropism which is also observed in sporophytes of ferns. Several hypotheses have been proposed to explain the mechanism for graviperception but none has been proven yet.

**Keywords** Slime molds · Fungi · Liverworts · Mosses · Ferns · Gravitaxis · Gravitropism

### 5.1 Introduction

Terrestrial organisms including fungi, mosses, ferns and higher plants use the gravity vector of the Earth to optimize their position in their environment (Blancaflor and Masson 2003). It is obvious that photosynthetic organisms orient their shoots upwards in order to maximize photosynthesis (negative gravitropism). Leaves or leaflets are often oriented at an oblique angle, which exposes the laminae perpendicular to the incident sun rays (Franklin and Whitelam 2004; Mano et al. 2006). Roots or rhizoids are usually oriented downwards (positive gravitropism or at an angle to the gravity vector to anchor the plant in the substratum (Cove 1992; Aloni et al. 2006). Even though fungi are not photosynthetic organisms, gravitropic orientation is beneficial to bring the fruiting bodies above the soil's surface and to facilitate the spreading of the spores.

As detailed in the introductory chapter of this volume, gravitropism—as well as gravitaxis in motile microorganisms—is based on a gravireceptor, a structure which senses the pressure of a heavy element, which, under the effect of gravity, exerts pressure on the sensitive element. The pressing element can be a statolith—a heavy organic or inorganic particle such as an amyloplast or an organelle containing barium-sulfate crystals (Sack 1997; Braun 2002). Alternatively, the whole cytoplasm of a cell

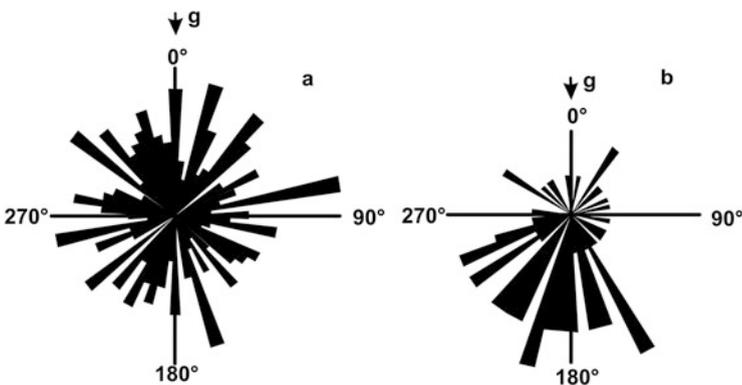
can exert pressure to an underlying membrane system. In some cases, mechanosensitive ion channels have been identified as gravisensors, while in other systems, the structural and biochemical identity still has to be provided.

Probably all organisms have developed the ability to sense the direction of the gravity vector of the Earth. Most are capable to respond to it with the exception of very small bacteria and viruses, which are subject to Brownian movement. However, the molecular mechanisms of graviperception may have been evolved several times.

## 5.2 Slime Molds and Fungi

The cellular slime mold *Dictyostelium discoideum* undergoes a life cycle, which includes a unicellular phase that consists of motile amoebae feeding on soil bacteria (Devreotes 1989). Under starved conditions some amoebae send out a cAMP signal, which is relayed through the population and results in a migration towards the signal center (Henderson 1975). There,  $10^3$ – $10^5$  amoebae aggregate to form a multicellular pseudoplasmodium (also called slug), which is about 0.5–2 mm long and 0.1 mm in diameter (Loomis 2012). The slugs are also motile and respond to a number of external stimuli including chemical clues, temperature gradients, light and gravity (Poff and Häder 1984; Häder and Hansel 1991; Maree et al. 1999; Song et al. 2006). They move for a few days until they culminate and form a sporangiophore with unicellular spores. Under optimal environmental conditions these spores are shed and form new motile amoebae.

When allowed to migrate on a vertical agar slab in a Petri dish the slugs showed a pronounced positive gravitaxis moving downward in contrast to the unicellular amoebae, which moved in random directions (Fig. 5.1). However, when a light stimulus was given simultaneously, gravitaxis was expressed only at low fluence



**Fig. 5.1** Circular histograms of the distribution and orientation of *Dictyostelium discoideum* amoebae (a) and slugs (b) on a vertical agar plate. 0° indicates top of the plate. Redrawn after Häder and Hansel (1991)

rates (Häder and Hansel 1991). The pseudoplasmodia are also remarkable since they show positive thermotaxis when exposed to a gradient of  $0.2\text{ }^{\circ}\text{C cm}^{-1}$  ignoring the gravistimulus. Irradiances  $> 10^{-3}\text{ W m}^{-2}$  canceled the response with respect to gravity.

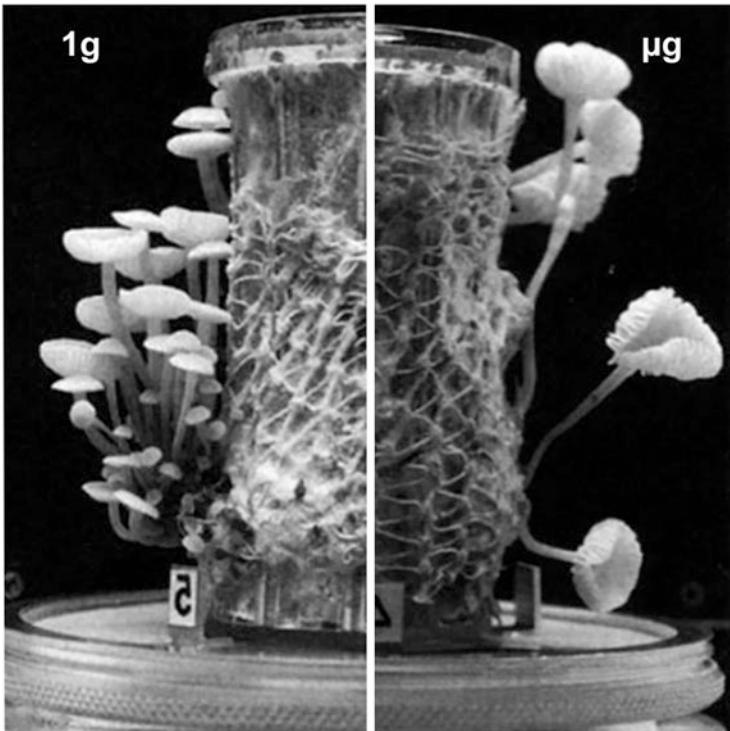
In contrast to the cellular slime mold *Dictyostelium*, *Physarum polycephalum* belongs to the acellular slime molds, which form undivided plasmodia with millions of nuclei (Guttes et al. 1961). The plasmodia consist of interconnected strands with a tough ectoplasm and a low-viscosity endoplasm which moves back and forth at regular intervals controlled by a  $\text{Ca}^{2+}$  oscillator (Coggin and Pazun 1996). When the shuttle streaming occurs more in one direction than in the other the whole plasmodium migrates in that direction. Also these myxomycetes respond to environmental stimuli including light, chemicals and gravity (Sauer 1982; Wolke et al. 1987; de Lacy Costello and Adamatzky 2013). On a vertical surface *Physarum* plasmodia show predominantly positive gravitaxis (68%), while 29% were indifferent and 13% showed negative gravitaxis (Wolke et al. 1987). When the vertical agar plate was rotated stepwise by  $90^{\circ}$ , the plasmodia reoriented accordingly. This graviresponse was investigated in real microgravity during the IML-1 mission on the Space Shuttle (Block et al. 1992) as well as under simulated microgravity on a fast rotating clinostat (Block et al. 1994). While no obvious statoliths can be detected in the plasmodia, mitochondria have been suggested to exert the pressure on a putative gravireceptor. It is interesting to note that the signal transduction chain involves cAMP which has been demonstrated in graviresponses of other organisms such as ciliates and flagellates as well (Block et al. 1998). Exposure to a few days of microgravity during a space experiment (STS-69) decreased the cAMP concentration in the plasmodia which was, thus, identified as a secondary messenger.

Fungal zoospores show a pronounced negative gravitaxis. The discussion, whether the spores of *Phytophthora* respond to an oxygen gradient, a light signal or a chemical clue, could be solved by proving that they reacted to gravity (Cameron and Carlile 1977). The cells also show chemotaxis, which was shown to be controlled by changes in the membrane potential, which regulates the flagellar activity; however, proof is still lacking, if the same mechanism is responsible for graviorientation (Cameron and Carlile 1980).

Mushrooms show a number of different responses to various environmental stimuli at different times of their development (Moore 1991). They display thigmotropism, gravitropism, anemotropism and phototropism. Young fruiting bodies grow perpendicular away from the substratum even in the presence of increased accelerations. Afterwards, the fruiting body shows positive phototropism and subsequently negative gravitropism; this shift is correlated with the initiation of spore formation. One interpretation for the onset of gravitropism is the assumption that the hymenia (forming to tubes or gills) in the fruiting body should be oriented vertically to facilitate the liberation of the spores falling downward in the free space. In addition, gravity is required for the morphogenesis of the fruiting body (Corrochano and Galland 2006). On an orbiting space station, *Polyporus brumalis* failed to initiate fruiting. Clinostat experiments also indicated that sporulation in *Lentinus tigrinus* and *P. brumalis* is prevented in simulated microgravity and karyogamy was

suppressed. When *Coprinus cinereus* was grown on a clinostat, it produced normal fruit body primordials but failed to produce spores. Even though the research on gravitropism in higher fungi (mushrooms) has been carried out for more than 125 years, the underlying mechanism still has to be revealed.

The fruiting bodies of the basidiomycete *Flammulina velutipes* show a clear negative gravitropic response. When oriented horizontally within 2 h the stems of the fruiting body respond with an increased elongation of the lower side, while it decreased by 40% in the upper side (Monzer et al. 1994; Kern et al. 1997). This growth response results in an overshoot, which is subsequently regulated. Under real microgravity in a space experiment, the fruiting bodies showed random growth orientation (Fig. 5.2) (Kern and Hock 1993). The graviresponsiveness seems to be restricted to the apical part of the stipe, which forms the transition zone to the pileus (cap or head). Light and electron microscopy showed that the hyphae in this zone are smaller and less vacuolated than in the basal part of the stipe. Complete removal of the pileus did not affect the gravitropic response, while excision of the transition zone abolished the gravitropic bending. In *Coprinus cinereus* gravitropic bending



**Fig. 5.2** *Flammulina velutipes*. Negative gravitropic orientation of *Flammulina velutipes* fruiting bodies grown for 5 days on Earth (1 g); fruiting bodies grown for 165 h in microgravity in Spacelab during the D2 mission show random orientation ( $\mu\text{g}$ ). Modified after Kern and Hock (1993)

starts even 30 min after the stems have been placed horizontally, however, only after completion of meiosis (Kher et al. 1992).

While several organisms have been found to use heavy statoliths or a heavy cytoplasm to exert pressure on an underlying gravireceptor, an opposite mechanism has been suggested for several fungi. In the zygomycete *Phycomyces blakesleeanus* some 200 lipid globuli are arranged in a spherical complex about 100  $\mu\text{m}$  below the growing tip of the vegetative sporangiophore (Grolig et al. 2004, 2006). The complex is held in place by a dense framework of filamentous actin. The buoyancy of these globuli could exert an upward pressure onto a gravireceptive structure. Experimental inhibition of the globuli formation by growing the sporangiophores at low temperatures reduces the gravitropic response. Similar lipid globuli have been found in the gravitropically responding hyphae of the fungus *Gigaspora margarita* and other fungi. In contrast, Eibel and coworkers suggest that gravitropism is instrumentalized by octahedral protein crystals with a specific mass of  $1.2 \text{ g cm}^{-3}$  located in the central vacuoles of the sporangiophore acting as statoliths (Eibel et al. 2000). Gravitropic mutants lack these protein crystals. Another publication offered a combined hypothesis based on both buoyant lipid globuli and sedimenting protein crystals. Molecular genetic approaches, magnetophoresis and laser ablation have supported the hypothesis that the actin cytoskeleton is involved in the gravitaxis and gravitropism sensory transduction chain (Kiss 2000).

### 5.3 Bryophytes

Dark-grown caulonemata and gametophores of the moss *Physcomitrella patens* show a pronounced negative gravitropism (Jenkins et al. 1986). After being placed horizontally, the caulonemata bend about  $20^\circ$  within 12 h and subsequently complete the  $90^\circ$  bending at a slower pace. Several mutants have been found which show a partially or completely inhibited gravitropism; one mutant even shows positive gravitropism. Negative gravitropism in wild-type protonema cells is reversed after a period of growth on a clinostat. The same reversal of the growth direction is observed during mitotic division (Knight and Cove 1991). Protoplast fusion resulting in somatic hybrids showed that at least three genes are involved in gravitropism. It is interesting to note that in none of these mutants gravitropism of the gametophores is affected indicating that the mechanisms of graviperception or transduction are different in caulonemata and gametophores. In contrast to caulonemata, rhizoids show a pronounced positive gravitropism and a negative phototropism (Glime 2017).

Amyloplasts have been discussed as possible statoliths in the protonemata of *Ceratodon purpureus* (Walker and Sack 1990). In the tip, there is a cluster of non-sedimenting amyloplasts with an amyloplasts-free zone below. The amyloplasts below this zone seem to be anchored by (actin?) filaments as they do not sediment to the basal wall, but to the lower cell wall in horizontal protonemata. This behavior

resembles that of the barium-sulfate filled statoliths in characean rhizoids. When placed horizontally, wild-type *C. purpureus* protonemata shortly bend downwards, which also occurs prior to cytokinesis (Wagner et al. 1997). UV-induced mutants also show negative gravitropism with kinetics similar to wild-type protonemata. Also *Funaria* caulonemata show upward bending (Schwuchow et al. 1995). The tip cells have a broad subapical zone, where plastid sedimentation has been observed. Under real microgravity on the Space Shuttle mission STS-87 and under simulated microgravity, protonemata cultures showed a radial outgrowth followed by a clockwise spiral growth (Kern et al. 2005). The protonemata of *C. purpureus* also show phototropism in red light. At irradiances  $\geq 140 \text{ nmol m}^{-2} \text{ s}^{-1}$  gravitropism was quenched, but amyloplast sedimentation was still observed (Kern and Sack 1999). These results show that both stimuli compete and that light regulates the gravitropic response.

In roots of higher plants, the gravitropic signal is relayed from the statenchyma in the root tip to the elongation zone via the plant hormone auxin, which is guided by several auxin transporters (cf. Chap. 6). Auxins and cytokinins have also been found in mosses and liverworts, where they regulate morphological development (Sabovljević et al. 2014). In *Marchantia polymorpha*, auxin is involved in the establishment of a dorsiventral polarity. Gemmae cups usually respond negative gravitropically, but after external application of auxin, they became positive gravitropic (Flores-Sandoval et al. 2015). These results indicate that the auxin regulation of growth and morphological development has been established in these earliest land plants.

## 5.4 Ferns

Trophophylls (sterile leaves) of the eusporangiate fern *Danaea wendlandii* show negative gravitropism, while sporophylls (fertile leaves) grow horizontally (Sharpe and Jernstedt 1990). This behavior has been attributed to a statolith mechanism since sedimenting amyloplasts have been detected in the cells of the petiole and rachis. Also dark-grown gametophytes of *Ceratopteris richardii* show negative gravitropism while light inhibits gravitropism (Kamachi and Noguchi 2012). Also in most *Selaginella* species light modulates the gravitropic response of rhizoids during the early developmental stage (Liu and Sun 1994). Later the effect of light is reduced while gravitropism dominates. Adult plants of the fern *Ceratopteris richardii* show pronounced graviperception and gravitropism. Single-celled spores of this fern were exposed to microgravity during a shuttle space flight (STS-93). cDNA microarray and Q RT-PCR analysis of spores germinating in microgravity showed significant changes in the mRNA expression of about 5% of the analyzed genes (Salmi and Roux 2008). Similar changes in gene expression are found in animal and plant cells.

## References

- Aloni R, Aloni E, Langhans M, Ullrich C (2006) Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. *Ann Bot* 97:883–893
- Blancaflor EB, Masson PH (2003) Plant gravitropism. Unraveling the ups and downs of a complex process. *Plant Physiol* 133:1677–1690
- Block I, Wolke A, Briegleb W, Wohlfarth-Bottermann KE, Merbold U, Brinckmann E, Brillouet C (1992) Gravitropism of *Physarum* – investigations in actual weightlessness. *Cell Biol Int Rep* 16:1097–1102
- Block I, Wolke A, Briegleb W (1994) Gravitational response of the slime mold *Physarum*. *Adv Space Res* 14:21–34
- Block I, Rabien H, Ivanova K (1998) Involvement of the second messenger cAMP in the gravity-signal transduction in *Physarum*. *Adv Space Res* 21:1311–1314
- Braun M (2002) Gravity perception requires statoliths settled on specific plasma membrane areas in characean rhizoids and protonemata. *Protoplasma* 219:150–159
- Cameron JN, Carlile MJ (1977) Negative geotaxis of zoospores of the fungus *Phytophthora*. *J Gen Microbiol* 98:599–602
- Cameron JN, Carlile MJ (1980) Negative chemotaxis of zoospores of the fungus *Phytophthora palmivora*. *J Gen Microbiol* 120:347–353
- Coggin SJ, Pazun JL (1996) Dynamic complexity in *Physarum polycephalum* shuttle streaming. *Protoplasma* 194:243–249
- Corrochano L, Galland P (2006) Photomorphogenesis and gravitropism in fungi. In: Kues U, Fischer R (eds) Growth, differentiation and sexuality. The mycota (A comprehensive treatise on fungi as experimental systems for basic and applied research). Springer, Berlin, pp 233–259
- Cove DJ (1992) Regulation of development in the moss, *Physcomitrella patens*. In: Brody S, Cove DJ (eds) Developmental biology. A molecular genetic approach. Springer, Berlin, pp 179–193
- de Lacy Costello B, Adamatzky AI (2013) Assessing the chemotaxis behavior of *Physarum polycephalum* to a range of simple volatile organic chemicals. *Commun Integr Biol* 6:e25030
- Devreotes P (1989) *Dictyostelium discoideum*: a model system for cell-cell interactions in development. *Science* 245:1054–1058
- Eibel P, Schimek C, Fries V, Grolig F, Schapat T, Schmidt W, Schneckenburger H, Ootaki T, Galland P (2000) Statoliths in *Phycomyces*: characterization of octahedral protein crystals. *Fungal Genet Biol* 29:211–220
- Flores-Sandoval E, Eklund DM, Bowman JL (2015) A simple auxin transcriptional response system regulates multiple morphogenetic processes in the liverwort *Marchantia polymorpha*. *PLoS Genet* 11:e1005207
- Franklin KA, Whitelam GC (2004) Light signals, phytochromes and cross-talk with other environmental cues. *J Exp Bot* 55:271–276
- Glime JM (2017) Ecophysiology of development: protonemata. In: Glime JM (ed) Bryophyte ecology. Michigan Technological University and the International Association of Bryologists, Houghton, MI
- Grolig F, Herkenrath H, Pumm T, Gross A, Galland P (2004) Gravity susception by buoyancy: floating lipid globules in sporangiophores of *Phycomyces*. *Planta* 218:658–667
- Grolig F, Döring M, Galland P (2006) Gravisusception by buoyancy: a mechanism ubiquitous among fungi? *Protoplasma* 229:117–123
- Guttes E, Guttes S, Rusch HP (1961) Morphological observations on growth and differentiation of *Physarum polycephalum* grown in pure culture. *Dev Biol* 3:588–614
- Häder D-P, Hansel A (1991) Response of *Dictyostelium discoideum* to multiple environmental stimuli. *Bot Acta* 104:200–205
- Henderson EJ (1975) The cyclic adenosine 3': 5'-monophosphate receptor of *Dictyostelium discoideum*. Binding characteristics of aggregation-competent cells and variation of binding levels during the life cycle. *J Biol Chem* 250:4730–4736

- Jenkins GI, Courtice GRM, Cove DJ (1986) Gravitropic responses of wild-type and mutant strains of the moss *Physcomitrella patens*. *Plant Cell Environ* 9:637–644
- Kamachi H, Noguchi M (2012) Negative gravitropism in dark-grown gametophytes of the fern *Ceratopteris richardii*. *Am Fern J* 102:147–153
- Kern VD, Hock B (1993) Gravitropism of fungi – experiments in space. Life sciences research in space, proceedings of the fifth European symposium. European Space Agency, Arcachon
- Kern VD, Sack FD (1999) Irradiance-dependent regulation of gravitropism by red light in protonemata of the moss *Ceratodon purpureus*. *Planta* 209:299–307
- Kern VD, Mendgen K, Hock B (1997) *Flammulina* as a model system for fungal graviresponses. *Planta* 203:23–32
- Kern VD, Schwuchow JM, Reed DW, Nadeau JA, Lucas J, Skripnikov A, Sack FD (2005) Gravitropic moss cells default to spiral growth on the clinostat and in microgravity during spaceflight. *Planta* 221:149–157
- Kher K, Greening JP, Hatton JP, Frazer LN, Moore D (1992) Kinetics and mechanics of stem gravitropism in *Coprinus cinereus*. *Mycol Res* 96:817–824
- Kiss JZ (2000) Mechanisms of the early phases of plant gravitropism. *Crit Rev Plant Sci* 19:551–573
- Knight CD, Cove DJ (1991) The polarity of gravitropism in the moss *Physcomitrella patens* is reversed during mitosis and after growth on a clinostat. *Plant Cell Environ* 14:995–1001
- Liu B, Sun G (1994) Effect of light on gravitropic response of rhizoids of gametophytes of ferns. *Wuhan Bot Res* 12:165–169
- Loomis W (2012) *Dictyostelium discoideum*: a developmental system. Elsevier, Groningen
- Mano E, Horiguchi G, Tsukaya H (2006) Gravitropism in leaves of *Arabidopsis thaliana* (L.) Heynh. *Plant Cell Physiol* 47:217–223
- Maree AFM, Panfilov AV, Hogeweg P (1999) Migration and thermotaxis of *Dictyostelium discoideum* slugs, a model study. *J Theor Biol* 199:297–309
- Monzer J, Haindl E, Kern V, Dressel K (1994) Gravitropism of the basidiomycete *Flammulina velutipes*: morphological and physiological aspects of the graviresponse. *Exp Mycol* 18:7–19
- Moore D (1991) Perception and response to gravity in higher fungi—a critical appraisal. *New Phytol* 117:3–23
- Poff KL, Häder D-P (1984) An action spectrum for phototaxis by pseudoplasmodia of *Dictyostelium discoideum*. *Photochem Photobiol* 39:433–436
- Sabovljević M, Vujičić M, Sabovljević A (2014) Plant growth regulators in bryophytes. *Bot Serbica* 38:99–107
- Sack FD (1997) Plastids and gravitropic sensing. *Planta* 203:63–68
- Salmi ML, Roux SJ (2008) Gene expression changes induced by space flight in single-cells of the fern *Ceratopteris richardii*. *Planta* 229:151–159
- Sauer HW (1982) Developmental biology of *Physarum*. Cambridge University Press, Cambridge
- Schwuchow JM, Kim D, Sack FD (1995) Caulonemal gravitropism and amyloplast sedimentation in the moss *Funaria*. *Can J Bot* 73:1029–1035
- Sharpe JM, Jernstedt JA (1990) Tropic responses controlling leaf orientation in the fern *Danaea wendlandii* (Marattiaceae). *Am J Bot* 77(8):1050–1059
- Song L, Nadkarni SM, Bödeker HU, Beta C, Bae A, Franck C, Rappel W-J, Loomis WF, Bodenschatz E (2006) *Dictyostelium discoideum* chemotaxis: threshold for directed motion. *Eur J Cell Biol* 85:981–989
- Wagner TA, Cove DJ, Sack FD (1997) A positively gravitropic mutant mirrors the wild-type protonemal response in the moss *Ceratodon purpureus*. *Planta* 202:149–154
- Walker LM, Sack FD (1990) Amyloplasts as possible statoliths in gravitropic protonemata of the moss *Ceratodon purpureus*. *Planta* 181:71–77
- Wolke A, Niemeyer F, Achenbach F (1987) Geotactic behavior of the acellular myxomycete *Physarum polycephalum*. *Cell Biol Int Rep* 11:525–528