

# CHAPTER 5-3

## ECOPHYSIOLOGY OF DEVELOPMENT: PROTONEMA

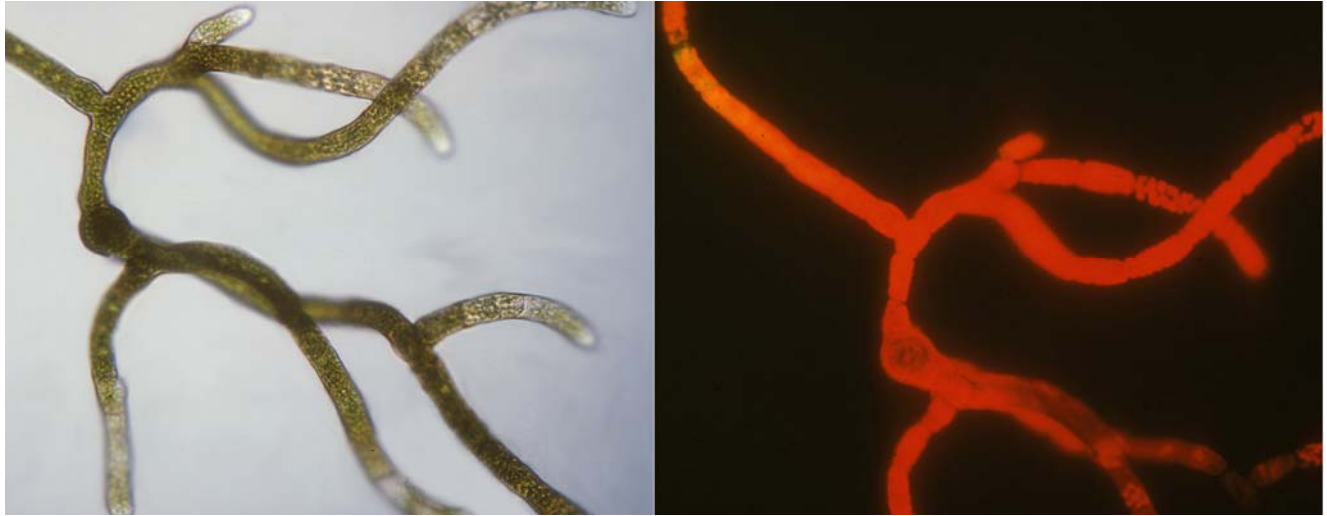


Figure 1. Protonema of *Fontinalis squamosa*. **Left:** white light. **Right:** UV light showing chlorophyll fluorescence. Photo by Janice Glime.

### The Protonema

The protonema is an elongate, thread-like structure that develops from the germinated spore of mosses and some liverworts. In most liverworts it is thalloid. The moss protonema typically branches (Figure 1) and can develop into **chloronema**, **caulonema**, or **rhizoids**, depending on the species and the conditions. The **chloronema** is the first thread formed by the germinating spore and is distinguished by its perpendicular crosswalls, short cells, numerous chloroplasts, colorless cell walls, and irregular branching. The **caulonema**, when present, develops later and is the source of gametophore buds in those species with both types of protonemal segments. It is distinguished by its distal position relative to the spore, longer cells with diagonal cross walls, usually brownish cell walls, and fewer, less evenly distributed, smaller, spindle-shaped chloroplasts. The chloronema, at least in culture, is able to grow vertically as well as horizontally, but the caulonema grows only horizontally (Bhatla 1994).

The protonemal stage is the best-studied part of bryophyte development. Due to its relative ease of culture and one-cell-wide structure, it has been the subject of numerous physiological studies to elucidate basic physiological mechanisms in plants.

As discussed earlier with life cycles, true moss spores germinate to form filamentous protonemata, but Sphagnopsida and Andreaeopsida form a thalloid protonema, and liverwort protonemata may range from filamentous to thalloid. Fulford (1956, in Watson 1974) identified 10 protonemal types in the leafy liverworts, but Nehira (1966) and Schuster (1966) warn us that the

protonema form is plastic and can be strongly modified by the environment. Nevertheless, Nehira (1966) identified 24 liverwort sporeling types.

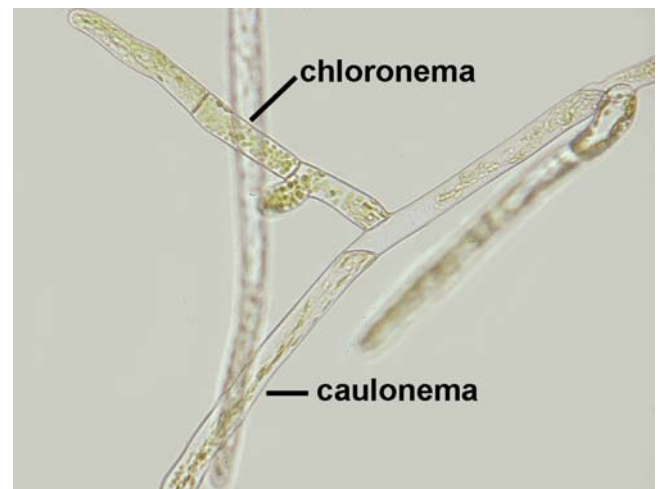


Figure 2. Distinction of chloronema and caulonema on the protonema of *Funaria hygrometrica*. Photo by Janice Glime.

Bittisnich and Williamson (1989) identified  $H^+$  efflux at the tips of the chloronema in *Funaria hygrometrica* and elaborated the role of acid flux in the extension of the protonema. However, unlike fungal hyphae, pollen tubes, and root hairs, the growth of the moss protonema is slow (Bhatla 1994) and is not confined to the apex. Growth apparently occurs in accordance with the acid growth

mechanism, in which  $H^+$  ions, induced by light and IAA, loosen the cell wall. In *Funaria hygrometrica*, acidification of the medium to pH 5.5 increases the extension of the tip cells, whereas buffering to a pH of 6.8 prevents it. Calcium seems necessary for the acquisition of new materials to the wall and the ability to extend the wall. When the leafy liverwort *Leucolejeunea clypeata* was grown on a Ca-free medium, the spores became distended, but the protonema failed to develop during the next five months of culture, whereas in the normal medium young plants had developed (Geldreich 1948).

The changes from distended spore to filamentous protonema growth to gametophore buds can require increasingly more specialized conditions. For example, Forman (1964) found that spore germination in *Tetraphis pellucida* requires a pH of 3.0-7.3 whereas growth of the leafy shoot occurs in the much narrower pH range of 5.1 to 5.8. Temperature requirements, on the other hand, are broader for the leafy shoot, but as the humidity drops, the viable temperature range narrows. Furthermore, the change from chloronema to caulonema can be delayed by inappropriate environmental conditions. Bopp (1961) found that the caulonema stage, and thus the bud stage, can be delayed by low temperature, submersion, or low light.

There seems to be controversy over the degree of difference between chloronema and caulonema, with Bopp (1959) contending that they are distinct stages, and Kofler (1958) and others finding no consistent distinction, even in *Funaria hygrometrica*, for which Bopp first made his claim. Several factors appear to lead to these disagreements (Watson 1974). The plasticity of the protonema permits it to respond differently to the varying environmental conditions. The distinction is exhibited more strongly in some species than others, and in some species, apparently no distinction exists. And, Kofler contended that genetic differences are more likely to be expressed in the protonema than in the gametophore or sporophyte because the environment has less time to exert selective pressure on the protonema. Hmmmm...

Even in mosses such as *Funaria hygrometrica* that have well-developed caulonema, culture in liquid media can inhibit the formation of caulonema, resulting in reduced bud formation – suggesting that very wet conditions would be detrimental to the development of gametophores in these taxa (Johri & Desai 1973). Furthermore, high cell densities cause failure of caulonema differentiation, suggesting some sort of self-inhibition. This might be another adaptive mechanism that prevents the gametophores from competing with each other and that permits the protonema time to revert to chloronema, spread to a wider area, or partially die off before putting forth upright plants.

Application of IAA induces the switch from chloronema to caulonema side branches (Johri & Desai 1973; Christianson 2000) and inhibits the further growth and initiation of chloronema branches (Johri & Desai 1973). Application of ABA to chloronema instead results in cell division and the formation of asexual reproductive cells, but not in caulonemata (Christianson 2000). Inadequate calcium causes the chloronema cells to divide unevenly and to form **tmema** (abscission cell that ruptures to release moss gemmae), but not in caulonemata. Cytokinin stimulates the formation of gametophore buds in

the caulonema, but not in the chloronema. Perhaps even more surprising, chloronemata exhibit positive phototropism, whereas caulonemata exhibit negative phototropism.

But are these hormone responses initiated by moss hormone productions? In the well-studied *Physcomitrella patens*, we do know that transition from chloronema to caulonema cells is under control of auxin (Gonneau *et al* 2001). Since IAA concentrations seem to be under environmental influence, variability and inconsistencies may be explained in the near future as we unravel the cryptochrome/IAA complex of reactions in this moss, and plants in general, using gene knockout techniques.

## Water Relations

We have often assumed that the protonema stage is the most susceptible to desiccation damage. However, this is not always true. During (pers. comm.) found that unsuccessful cultures of xerophytes such as *Grimmia* produced gametophores only after being put aside and forgotten, *i.e.*, after desiccation. But it is surprising that Glime and Knoop (1986) found that after cultures of the aquatic moss *Fontinalis squamosa* had dried out, added water caused the protonemata to swell and again become active. This is further supported by observations on protonemata that dried overnight on a microscope slide. When I added water to observe them for fluorescence, they produced vivid red chlorophyll fluorescence and regained their normal shape. It appears that protonemata may have considerable desiccation tolerance.

Further evidence that the protonema is desiccation tolerant can be gleaned from their dispersal period. As seen in the chapter on phenology, dispersal in spring is commonplace. It would seem, therefore, that the protonema must be growing in summer, when desiccation is most likely. The other period of high spore dispersal is fall, again preceding the dry season of winter. Although we have insufficient evidence to show that the protonemata are present during these two relatively dry seasons, it appears likely that they are in at least some, if not many, species. Figure 3 shows a hydrated protonema in the field.

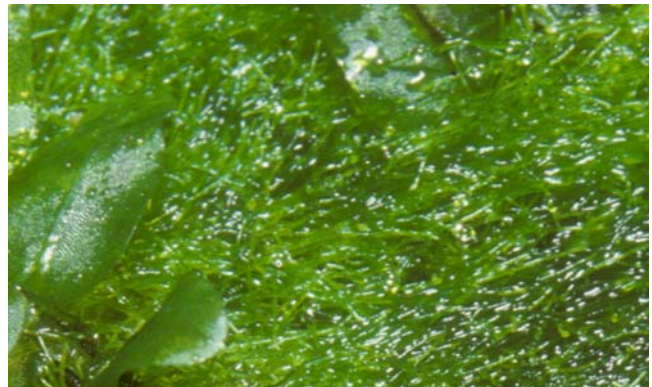


Figure 3. Protonema of *Plagiommium* in the field. Photo by Janice Glime.

## Seasonal Light/Temperature Changes

It is hard to talk about light without also considering temperature, since brighter light generally means greater exposure and higher temperatures. Higher temperatures and brighter light are also usually coupled with a longer

photoperiod. Knowledge of their effects on protonemal growth and development is based on laboratory cultures.

Light, coupled with temperature, seems to play a role in the pattern of development of protonemata in the aquatic moss *Fontinalis*. *Fontinalis squamosa* spores germinated throughout the range of 40 to 3000 lux, and cultures exhibited unipolar, bipolar, tripolar, and one tetrapolar germination (Glime & Knoop 1986). The number of germ tubes was generally consistent within a single plate, despite having bands of spores from three different capsules. At 3°C and 120 lux, germination required four weeks, and only distended spores with a single protrusion were present (Figure 4). At 14°C, 1200 lux, two plates of spores had single threads (Figure 5), one had double threads, and one had short single and double threads. At 20°C, 2100 lux, two plates had only single germ threads that formed weak spirals and two had many spores with two or three germ threads and no spiral growth (Figure 6); branching was much more extensive than at 14°C and 1200 lux. Although effects of temperature cannot be separated from those of light intensity, they mimic environmental conditions as they change from winter to summer. Such environmental controls can prevent spores from germinating or protonemata from developing too early in the season. The high degree of branching at higher light and temperatures could afford more self-protection from desiccation by providing overlapping threads. Bipolar and tripolar germination is also likely to be a response to the greater ability to photosynthesize with more light and provide energy for the developing germ tube.

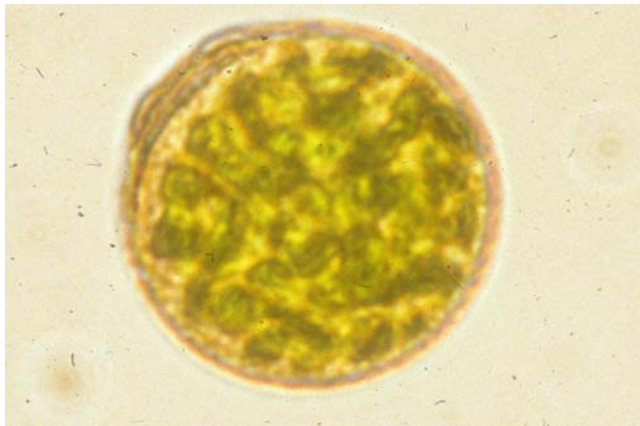


Figure 4. Distended spore of *Fontinalis squamosa* as one might find at 3°C and 120 lux. Photo by Janice Glime.



Figure 5. Single-thread protonemata of *Fontinalis squamosa* formed at 14°C and 1200 lux. Photo by Janice Glime.

High temperatures required for the protonemata can force a species into a narrow geographic range despite the ability of the spores to germinate at cooler temperatures. For example, *Anisothecium molliculum* has an optimum temperature of 25°C, not only for protonemal growth, but also bud formation (Kumra & Chopra 1985), preventing it from living in polar regions.

At least for *Fontinalis squamosa*, higher light intensity and temperatures result in more germ tubes arising from the spore, suggesting that more sugars might be available, both for energy and for creating a high osmotic potential. The increased number of protonematal branches at higher light intensities and temperatures could provide a thicker mat to decrease evaporative losses and to increase self-shading against UV light damage.

### Light Intensity

High light intensity can promote protonemal growth, as in *Microdus*, *Hymenostylium*, and *Campylopus* (Mehta 1988). In the ephemeral *Physcomitrella patens*, high light intensities promote branching of the caulonema, thus proliferating the potential bud sites (Cove *et al.* 1978, 1979). Continued high light promotes secondary caulonemata instead of bud formation. Is this adaptive by extending the plant to a darker location? Or is it merely a way of measuring all the available illuminated space for successful gametophores? Sood (1975) also observed an effect of light intensity on the number of germ tubes arising from the spore in *Pogonatum aloides*. At 1000 lux germination was unipolar, increasing at 3000 lux. At 6-8000 lux some spores swelled but failed to germinate. In germinating spores of *Polytrichum commune* and *P. juniperinum*, there was a lag in synthesis of chlorophyll, being longer in *P. commune* (Karunen 1973). The chlorophyll *a/b* ratio at that time in *P. commune* was 1.4-1.8, thus providing little antenna effect by chlorophyll *b*. The low concentration of chlorophyll in general and the reduced relative amount of light-gathering chlorophyll *b* would force the gametophyte to require food reserves during early development.

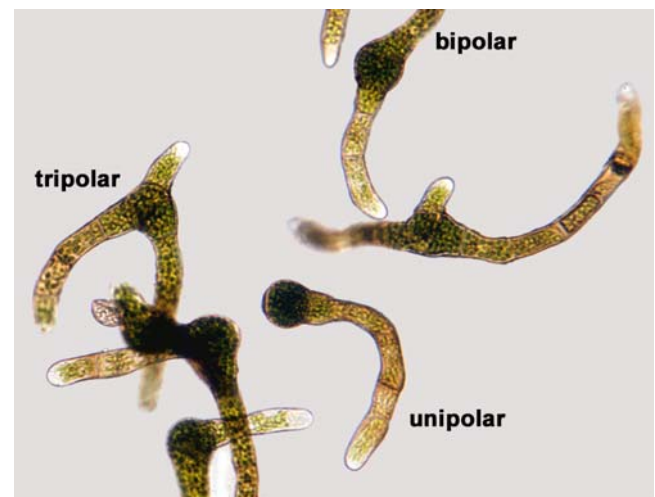


Figure 6. Protonemata of *Fontinalis squamosa* showing unipolar, bipolar, tripolar germination typical at 20°C and 2100 lux. Photo by Janice Glime.

Although light generally seems to be necessary for spore distension, in some cases the protonema can even grow in the dark. In *Ceratodon purpureus* darkness first induces an increase of starch grains in the chloroplast (Valanne 1971). This is followed by disappearance of starch and an increase in the number of grana lamellae.

## Light Quality

It is clear that light quality affects the growth and development of at least some protonemata. Light quality shift from white light to green and far red, as found in the forest, resulted in reduced protonemal growth in *Pohlia nutans*, with the least growth occurring in green light (Mitra *et al.* 1959). Giles and von Maltzahn (1967) found that red light stimulates mature leaf cells of *Plagiomnium affine* to regenerate by protonemata, and they suggested that phytochrome was most likely involved. Although liverworts seem to lack any consistent kind of photoregulation (Hartmann & Weber 1990), mosses respond differently to different wavelengths. Their best chloronema growth seems to be in white light (Bhatla 1994), but we must question whether this is true for all species that grow only under a canopy of green. In *Funaria hygrometrica*, the red range stimulates normal growth, whereas the blue range leads to the development of caulonema-like cells. It is possible that these shifts in light quality response could help to signal the time to develop gametophores as the protonemal mat thickens from extensive growth, changing the light quality of underlying strands.

Light quality could also serve to signal that it is time to break dormancy. Both blue and red light will permit maintenance of normal chloroplasts in *Ceratodon purpureus* protonemata, but blue light results in richer starch, denser stromata (colorless matrix of chloroplast in which packets of chlorophyll are embedded), and more mitochondria, whereas red results in a more effective use of lipids (Valanne 1971). Is there any adaptive value in this? Is the moss able to sense the decreasing cover by snow, as voles do, based on light quality and intensity?

## Photoperiod

In *Ceratodon purpureus*, long days stimulate elongation of the protonema, whereas short days result in protonemal branching (Larpen-Gourgaud & Aumaitre 1980). The two systems are antagonists. This relationship suggests that an IAA/cytokinin balance may be the important controlling factor, with long days promoting IAA, probably through phytochrome mediation.

In *Bryum pseudotriquetrum* a day length of ten or more hours is required for germination and protonema growth (Kinugawa & Nakao 1965, Figure 7). However two minutes of light during a 16-hr dark period is sufficient to remove the inhibitory effect developed during the dark period and will likewise stimulate germination and growth. This further supports the hypothesis of a phytochrome response and is much like the photoperiodic control of flowering.

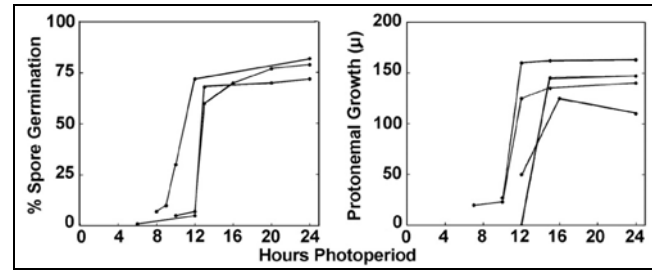


Figure 7. Effect of photoperiod on spore germination after 5 days (left) and protonema growth after 3 days (right) of *Bryum pseudo-triquetrum*. Redrawn from Kinugawa & Nakao (1965).

## Hormonal Response

The complexity of these light responses and the implications of involvement by phytochrome is undoubtedly under the control of hormones. In the ephemeral *Physcomitrella patens*, light and hormonal combinations coordinate development (Cove *et al.* 1978, 1979). Cytokinin in the presence of auxin promotes buds (Gorton & Eakin 1957), and high concentrations inhibit caulonemata (Cove *et al.* 1978, 1979). This combination would therefore promote caulonema growth while the caulonemata are sparse, ensuring sufficient plants for a viable population and providing a sufficiently dense protonemal mat to help maintain moisture at the soil surface. When this mat becomes very dense, self-shading could stimulate the production of auxin and cytokinin and shift the development to bud formation. Once these self-shaded protonemata have shifted to bud development, they are likely to communicate this signal to the surface protonemata and induce buds throughout the mat. Figure 8 shows a developmental scheme modified from Cove *et al.* (1979) to include these environmental stimuli.

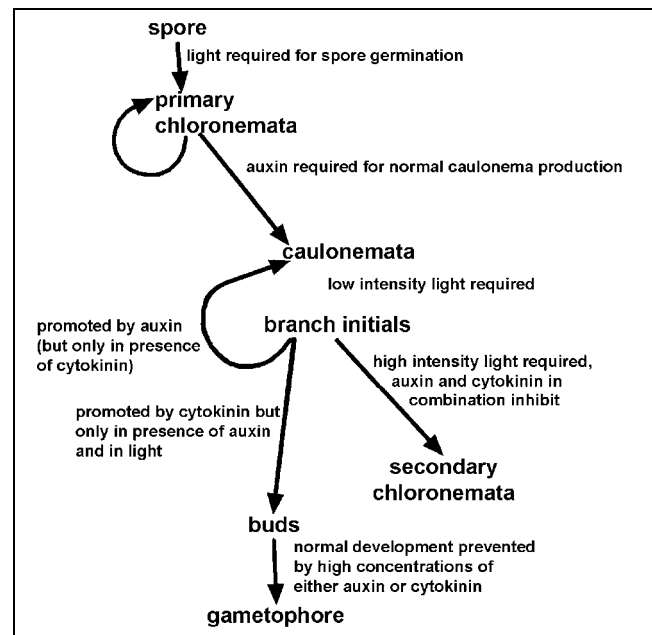


Figure 8. Effects of auxin and cytokinin on *Physcomitrella patens*. Redrawn from Cove *et al.* (1979).

Bierfreund *et al.* (2003) used *Physcomitrella patens* to determine the distribution of auxin in the protonema. As in higher plants, the highest concentrations were in the dividing and young cells. Concentrations declined from the

tip cells back to the basal cells of the protonema, supporting earlier work of Bopp and Atzorn (1992).

Auxin (IAA) is important in the transition of chloronema to caulonema (Johri & Desai 1973; Figure 8) and the appropriate concentration maintains the caulonema state (Bopp 2000). Although we generally think that endogenous hormones from one plant cannot affect another, in *Funaria hygrometrica* the minute quantity of  $10^{-16}$  mol IAA/mg fw seems to be responsible for the change from chloronema to caulonema (Bhatla & Dhingra-Babbar 1990). Such a small quantity could surely leak from other members of the same species or from a different species to help coordinate behavior among individuals. In fact, as the protonema matures, the protonema can excrete most of its auxin to its substrate, as shown in *Physcomitrella patens* (Reutter *et al.* 1998).

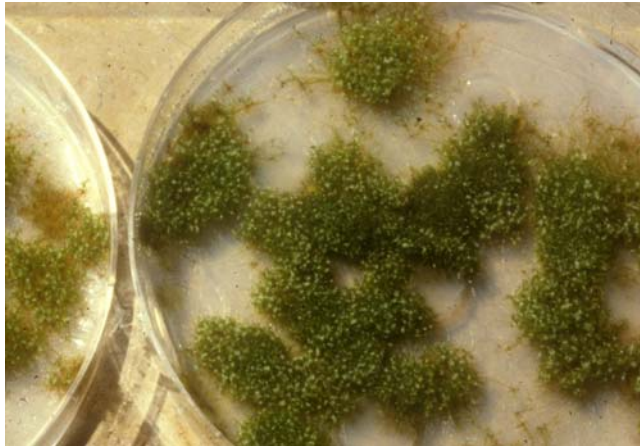


Figure 9. Culture of *Funaria hygrometrica* showing distinct colonies resulting most likely from hormonal interaction between clones at the protonemal stage. Photo by Janice Glime.

We already know that uptake of IAA by the protonema occurs; in the lab, uptake of IAA by protonematal cells is both passive and active. The passive component is pH-dependent, with the greatest increase in uptake occurring at pH 4.5-4.7, indicating a dissociation of the IAA molecule ( $pK = 4.7$ ;  $pK$  is pH at which equal concentrations of acidic and basic forms of substance are present). The potential for an exogenous developmental regulator has enormous implications not only for development, but for systematics and ecology as well.

## Tropisms

**Tropisms**, the bending of a plant in response to a stimulus, are adaptive in orienting the plant into its most beneficial position. In bryophytes, protonemata are **positively phototropic** (bend toward light), whereas **rhizoids** are **photonegative** (bend away from light) (Heitz 1942). Although Kofler and coworkers investigated the effects of the environment on bryophyte tropisms as early as 1958 (Kofler 1958, 1971; Kofler *et al.* 1963), bryophyte tropisms have remained largely unstudied until recently. However, because of their simple protonemal structure, much of our current understanding of tropisms in plants has been learned from using bryophytes as model systems. The one-cell-thick protonema makes it easy to observe the **statoliths** that are special **amyloplasts** (colorless plastids containing starch) that respond to gravity. These statoliths are involved in **gravitropism** (directional growth in

response to gravity). The ability to knock out or add genes that are easily expressed in the  $1n$  plants has made the necessary manipulation much easier than in tracheophytes.

Yet bryophytes have different **phototropic** responses (directional growth in response to light) from those of tracheophytes. Rather than responding to blue light, as do the tracheophytes, most bryophytes seem to respond to red light, using phytochromes instead of cryptochromes as their sensory pigments (Wada & Kadota 1989). Jaffe and Etzold (1965) demonstrated that even spores in *Funaria* respond to red light, resulting in chloronema growth in the opposite direction from that of rhizoids. And even more intriguing is the ability of bryophytes to store a phototropic stimulus (Hartmann & Weber 1988), further suggesting the use of phytochromes. However, the expected dark reversal does not occur, indicating something else is involved (Christianson 2000).

Schwuchow and Sack (1990) reported for the first time an effect of gravity on **microtubule** (essential protein filament of cell structural skeleton) distribution in plants, based on studies in protonemata of *Ceratodon purpureus*. In fact, this moss served as the model organism to demonstrate that microtubules help organelles to maintain their positions within the cell (Schwuchow & Sack 1994). Nevertheless, our understanding of **gravitropism** in protonemata is still in its early stages. We don't even have a very long list yet of mosses with demonstrated protonemal gravitropism, and we seem to know even less about liverworts. Schwuchow *et al.* (2002) have only recently found tropisms in *Barbula unguiculata*, *Fissidens adianthoides*, *Fissidens cristatus*, and *Physcomitrium pyriforme*, despite the report of positive phototropism in *Funaria* protonemata in 1942 by Heitz.

Repp *et al.* (2004) used genetically modified *Physcomitrella patens* to demonstrate the role of cytokinin signalling for gravitropism. When a knockout mutant lost its sensitivity to cytokinin, it had greatly reduced ability to respond gravitropically in the dark.

Wagner and Sack (1998) reported that the gravitropic response occurs within 1-2 cell divisions in the protonemal tip cells of *Ceratodon purpureus*, which grow upward in the dark (Wagner *et al.* 1997). These five mosses and four other species, representing five orders, support the hypothesis that amyloplast sedimentation probably serves in gravity sensing in moss protonemata. It appears that these amyloplasts tug on the **cytoskeleton** (structural support within cell), pulling down on it, much like trapped insects on a spider web. One theory is that this causes the cytoskeleton to pull on the cell membrane, creating larger holes in the membrane that facilitate the entry of  $Ca^{++}$ . This creates a higher concentration of  $Ca^{++}$  on the upper side of the cell, possibly causing it to inhibit the IAA on that side of the cell. Auxin inhibitors block this gravitropic response in *Ceratodon purpureus* (Schwuchow *et al.* 2001), suggesting the role of IAA in the process. Nevertheless, high levels of exogenous IAA do not block the apical tropism of the protonema, suggesting the mechanism differs from that in higher plants.

What little we thought we knew about gravitropisms in moss protonemata was further confused when growing protonemata of the moss *Ceratodon purpureus* took a two-week trip in space on the space shuttle Columbia (Miller & Phillips 2003; Kern *et al.* 2005). On 16 July 2002, plant

physiologist Fred Sack carefully opened a Petri dish that had spent the two weeks without gravity and without light. To his surprise, the protonemata had grown in a spiral pattern (Figure 10). This is quite different from the normal tangle of protonemata grown on Earth.

According to Fred Sack (Miller & Phillips 2003), "These odd spirals mark the first time in space that a plant normally oriented by gravity has grown in a non-random



Figure 10. Spiral growth of the protonemata of *Ceratodon purpureus* aboard the space shuttle Columbia. Photo courtesy of Fred Sack.

Another piece of this gravitropic puzzle is that a high-gradient magnetic field can substitute for gravity, causing curvature of tip cells in *Ceratodon purpureus* (Kuznetsov *et al.* 1999). Genetically modified protonemata with larger plastids responded more strongly, supporting the hypothesis that plastids are involved in gravity sensing.

It appears, based on our observations with protonemata, that the statoliths (amyloplasts) settle downward within the cell in response to gravity. This pulls on the cytoskeleton. The cytoskeleton is attached to the cell membrane, so this downward pull tugs on the membrane in the upper portion of the cell (Figure 11). A plausible theory is that this stretches the membrane, making it more permeable. This in turn permits more  $Ca^{++}$  to enter the upper side of the cell, where it inhibits the action of IAA, permitting the lower side of the cell to grow more.

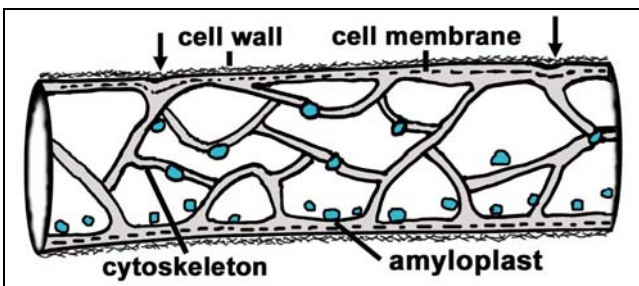


Figure 11. Schematic model of hypothetical relationship of amyloplasts (statoliths) of a protonema in response to gravity. Arrows denote pull of cytoskeleton on cell membrane. Drawing by Janice Glime.

pattern." The puzzle begins with the **amyloplasts**. These starch bodies experience sedimentation in gravity and seem to tug on the cell skeleton. However, on the shuttle, with no gravity, this should not happen. Rather, they should float at random within the cell. Instead, they bunched together. This indicates a natural propensity for growing in a spiral that is overridden by the gravity of Earth. Perhaps Seifritz was right – all life does have a twist in it.

## Nutation

Under some circumstances, the protonema will exhibit **nutantion** – a spiral or circular growth pattern that is displayed in time-lapse photography by apparent movements of the stem (or protonema) in a circle. In *Funaria hygrometrica*, red light causes the protonema to grow into a ring (Simon & Naef 1981). I have observed the same nutation in contaminated cultures of *Fontinalis squamosa* and in air-grown rhizoids of that species. Nutation appears to facilitate a kind of seeking – altering growth directions until a more favorable condition is located. It is beneficial when no directional stimulus is present, such as spiral growth of rhizoids until they contact a substrate, as observed in *Fontinalis squamosa*. Although nutation is an IAA/ethylene response in higher plants (Morgan & Powell 1970), its occurrence as a response to red light suggests it results from a somewhat different mechanism here since red light is known to inhibit ethylene production. Could this be the same spiraling mechanism seen in the space-travelling *Ceratodon purpureus* protonemata? The curiosity there is that the entire population of protonemata grew in a spiral.

## Interactions

We have already implied that exogenous growth regulators could determine events in the development of the moss protonema. Protonemata grow on substrata that are not sterile. Rather, they are teaming with fungi, bacteria, algae, and exudates of other plants. One might then predict that at least some of the protonemata respond in positive or negative ways to these companions.

One possible outcome of cohabitation is that fungi or other organisms may provide the growth substances needed to stimulate the next phase of development. Fungi commonly produce gibberellic acid that escapes into the environment. Vaarama and Tarén (1059) found that not only did 0.01% GA promote both spore germination and protonema growth in several mosses (*Dicranum scoparium*, *D. undulatum*, *Dicranoweisia crispula*, and *Pogonatum urnigerum*), but also inoculation with several fungi (*Aspergillus flavus*, *Penicillium martensii*, *Mucor racemosus*, *Fusarium scirpi*, & *Rhodotorula ad mucilaginoso*) had even more effect than did the gibberellic acid.

In contaminated cultures of *Fontinalis squamosa*, most of the protonemata formed mature caulonemata in less than four weeks, whereas in uncontaminated cultures the chloronema state predominated (Glime & Knoop 1986; Glime, unpub data). And only the contaminated cultures ever produced buds. This suggests that at least some microbes might alter the developmental state of the moss. Based on these observations and those of Spiess *et al.* (1971) on the influence of bacteria on the development of *Pylaisiella*, it appears that we should focus some of our

attention to possible developmental interaction with the microbial community.

Fungi have effects on other bryophyte protonemata as well. Hildebrand and coworkers (1978) found that fungal exudates promoted the growth of *Atrichum*, *Funaria*, and *Brachythecium* protonemata at low pH. As suggested above for spore germination, *Splachnum ampullaceum* (Figure 12) protonematal growth is promoted by several species of fungi (von Maltzahn & MacQuarrie 1958). Certainly growth hormones exuded by the fungi could be of importance here (see Bopp 1980).



Figure 12. *Splachnum ampullaceum* growing among *Sphagnum* on dung, where changing dung conditions and fungal exudates influence development. Photo by Janice Glime.

In addition, contributions of vitamins from algae or amino acids or other organic compounds from bacteria might either be essential or promote a growth rate that is compatible with the seasons. Gibberellic acid, produced by many fungi, has a variety of effects, depending on the species of moss. It increases the number and length of protonemal cells in *Dicranum* and *Dicranoweisia*, but it has no effect on *Racomitrium fasciculare* (Vaarama & Tarén 1959). Since *R. fasciculare* grows on rocks where fungi are unlikely to occur, and fungi are a natural source of GA, these differences in responses are consistent with habitat differences.

We know that the induction Factor H (an adenine derivative discussed earlier) is present in *Funaria*. It will induce not only other protonemata of *Funaria*, but it can be induced by other species (e.g. *Leptobryum pyriforme*) as well (Klein 1967; Bopp 1976). Such a factor is adaptive in insuring a sufficient breeding population, but perhaps more importantly it insures a community organization that offers resistance against desiccation, where middle plants are protected by outer ones in the population. In submerged mosses such as *Fontinalis*, on the other hand, moisture conservation is not so critical, and multiple gametophores would only offer competition for the limited substrate available for anchorage.

Whereas some interactions can enhance growth of moss protonemata, others inhibit it, preventing the colonization of that substrate. Shrimal (1975) showed that bark extracts of several trees inhibited mitosis in onion root tips and caused non-separation of chromosomes. If these substances have the same effects on mosses, it could explain why some trees lack bryophytic epiphytes.

Inhibition can also occur within a species, as already suggested for *Funaria*. In this species, protonemata from

several spores in one culture will not intersect (Watson 1981). The mat attains the same density when the protonemata are derived from many spores as when they are derived from only one. Watson also suggests that one species may inhibit another, thus making time an important factor in access to a habitat. And *Funaria* is not the only moss where some exudate of the protonema retards development of competing protonemata of the same species. This has been observed in culture in *Physcomitrella patens* as well (Schween *et al.* 2003). It is perhaps a widespread phenomenon.

In *Funaria*, this factor of inhibition seems to break down in mature cultures. When I placed disks of agar from a mature culture onto fresh plates and inoculated the plates with spores, some of the protonemata grew on the disks from the mature cultures. In no case did I find a zone of inhibition around the agar disk. This suggests to me that the substance preventing live protonemata from intersecting might be a gas produced by the growing protonemata. Gases are instrumental in maintaining maximum distance among sporangia of some slime molds, and one gas that could accomplish this in mosses is ethylene. Since ethylene is known from *Funaria* protonemata (Rohwer & Bopp 1985) and it is a known inhibitor of cell division (Abeles 1973), small concentrations produced by the tips could easily signal their presence to neighbors. Ethylene production is stimulated by the action of IAA on S-adenosylmethionine (SAM), so we might expect the tip (where there is the most IAA) to have the highest ethylene concentration. The longest branches will interact first, and these are the ones most likely to be IAA-rich and apically dominant.

Hormones produced by other organisms in the environment can affect the development of protonemata, and in some cases these may be required to take the bryophyte to its next developmental stage. Among these, GA (gibberellic acid) is a likely candidate. It is produced by many fungi and readily enters the environment. It is known to increase the number and length of protonematal cells in some soil-inhabiting species, but may have no effect on rock-dwelling taxa that normally would have much less contact with soil fungi. Bark exudates may also inhibit growth of some bryophyte protonemata, and some bryophytes may inhibit each other, both of different species and of other clones of their own species.

## Nutrients

In some mosses, the form of the protonema is dependent on available nutrients. For example, in nature *Sphagnum* normally has a thalloid protonema (Figure 13). However, in a medium with high potassium, the protonema becomes filamentous (Schofield 1985). Since *Sphagnum* normally grows in habitats very low in potassium, this filamentous growth form is not observed in nature.

Sundberg and Rydin (2000) showed that *Sphagnum* establishment from spores was limited by amount of phosphate released by underlying litter. Added moose dung likewise promoted establishment. They concluded that cover of other plants and nutrient release from litter provided safe sites where *Sphagnum* spores could germinate and establish new plants.

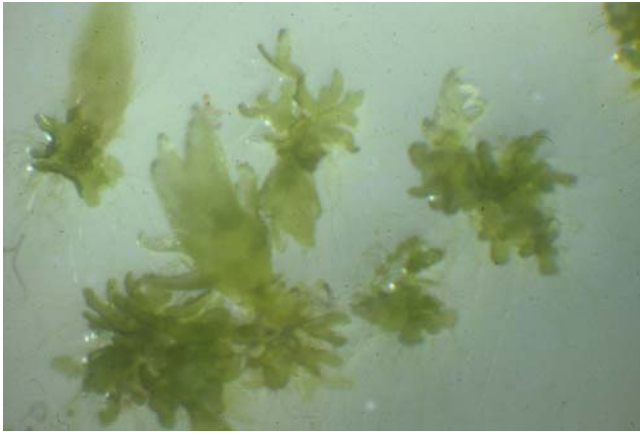


Figure 13. Thalloid protonemata of *Sphagnum papillosum*. Photo by Yenhung Li.

Various heavy metals seem to alter protonematal form. Kapur and Chopra (1989) found that in the moss *Timmiella anomala*, when grown aseptically (conditions free of microorganisms), aluminum causes protonemal cells to become rounded and packed with chloroplasts and starch grains; the filaments themselves form bunches. Zinc and arsenic likewise cause rounded cells, with zinc-damaged cells becoming reddish; most arsenic effects are seen at the terminal and intercalary positions. Mercury causes cells to become broad with dense particles, whereas nickel results in long, thin protonemata with little branching. At  $10^{-6}$  M, nickel increases protonemal growth slightly, but at  $10^{-5}$  M it drastically decreases the number of gametophore buds. Cobalt inhibits protonemal growth but seems to have no effect on bud formation. What do these effects mean to development of the moss, and are they likely to occur in nature where soil chelators (organic compounds that bind metal by forming ring structure around it) may inhibit uptake, or concentrations never reach these levels? Could they actually affect appearance of mature gametophytes resulting from these anomalous forms and hence confound our understanding of the taxonomy?

Calcium seems important to protonema development in some species and may be the actual factor where pH affects viability. For *Funaria hygrometrica*, Reiss and Herth (1979) suggest that a calcium gradient is responsible for protonemal tip growth. The calcium concentration is highest at the tip where fluorescence is strongest. It is likely that calcium is involved in transport of substances across cell membranes.

Nutrient availability is affected by pH. Thus pH could affect success of protonemata. In *Physcomitrella patens* (Figure 14, Figure 15), changes in pH in the range of 4.5 to 7.0 influenced differentiation of protonemata but did not have any negative impact on growth rate (Hohe *et al.* 2002). In another example, *Anisothecium molliculum* has an optimum pH of 5.5 for not only protonemal growth, but also for bud formation (Kumra & Chopra 1985). The pH may not only alter the ability of bryophyte protonemata to obtain nutrients, but also affect their susceptibility to exudates from other plants and fungi. Following fire, invasion by bryophytes onto the charred substrate seems to be likewise influenced by both pH and residual chemicals (Thomas *et al.* 1994). Germination success in the moss *Campylopus pyriformis* is positively influenced by increases in the pH in the range of 3.5-6.4.



Figure 14. *Physcomitrella patens* in its natural habitat where pH and moisture can change considerably as spring flooding recedes. Photo by Michael Lüth.



Figure 15. *Physcomitrella patens* plants with protonemata on the wet soil. Photos by Michael Lüth.

Our knowledge of nutrient requirements is based mostly on cultures of bryophytes and we know little of the generalities that might be important. For example, elevated potassium causes *Sphagnum* protonemata to become filamentous instead of thalloid, but in nature we have not observed protonemata in habitats where this condition exists. The level of phosphorus is often limiting and we can assume this plays a role in nature as well. An important observation is that heavy metals such as aluminum, zinc, mercury, and arsenic can cause abnormal protonemata with such symptoms as rounded cells with dense chloroplasts and starch. Elevated nickel, on the other hand, causes the protonemata to be thin. Calcium is undoubtedly important and its function may relate to membrane transport of other ions into the cell. All of these nutrient effects are likely to be affected by the pH because a lower (acidic) pH generally makes most nutrient ions more soluble.

## Rhizoids

Rhizoids form on the protonema at different stages, depending on the species and the growing conditions. On nutrient-free agar and in distilled water the first filaments to emerge from the spore are rhizoidal (Bhatla 1994). They are distinguished by their pigmented (usually brown) cell walls, oblique crosswalls, and discoid or cylindrical plastids. The rhizoids seem to depend on forced calcium entry (active uptake requiring energy) for growth and at least in those tested, respond positively to a calcium gradient (Bhatla 1994).

Rhizoids usually exhibit strong positive **gravitropism** (grow toward the center of gravity), negative **phototropism** (grow away from light), and **thigmotropism**

(alter their growth upon contact), with the latter overriding the effects of the former once a substrate is contacted (Bhatla 1994). When growing in air, they often exhibit a spiral growth (**nutation**) until a substrate is contacted (Glime 1987). Upon contact, they may branch into short, fingerlike tips (Odu 1988), as noted in *Lophocolea cuspidata* (Odu & Richards 1976) and *Fontinalis squamosa* (Glime 1987). Among the liverworts, apical branching seems to be in part phylogenetically constrained, appearing commonly in the Jungermanniales but only in the Metzgeriineae of the Metzgeriales and not at all in the Marchantiopsida (Pocock & Duckett 1985). Those liverworts with swollen rhizoids grow exclusively on peat and rotten wood associated with fungal hyphae. Pleurocarpous moss rhizoids become flattened near the tips, but in acrocarpous mosses these flattenings extend well behind the tips of the rhizoids (Odu 1988).

Adhesion of rhizoids seems to be stimulated by the substrate itself (Odu 1988). Upon contact, rhizoids produce such extra-wall materials as sulfated mucopolysaccharides. These are highly viscous substances that serve as a sticky adhesive, also known in algae and other microorganisms.

But what controls the production of these rhizoids? Goode *et al.* (1992) were unable to get *Tetraphis pellucida* to produce any protonemal rhizoids in culture, yet these occurred routinely in nature. They ascribed this difference to the limited nutrients and different irradiance in the wild. But hormones available from surrounding vegetation, bacteria, and fungi could play a role as well, as they apparently do for the protonemata.

## Tmema

**Tmema** cells are rounded cells that rupture, setting free a protonemal gemma. These cells result from a very unequal division of the cell near the proximal cross wall and divide the chloronema filaments into fragments of only a few cells. The tmema cells have few chloroplasts which soon become reduced in size, but the cell elongates in its proximal direction by expanding its newly formed wall, progressing in the opposite direction from normal cells. The new tmema wall forms inside the old lateral wall and the subsequent loosening of the old wall results in fragmentation of the protonema. This separation also occurs in older, untreated cultures of *F. hygrometrica* (>25 days) (Bhatla & Dhingra-Babbar 1990).

Bopp and coworkers (1991) have demonstrated the importance of IAA in the development of **tmema** cells in protonemata of *Funaria hygrometrica*. They found that addition of only 10  $\mu$ M IAA would suppress the production of tmema cells, indicating IAA deficiency. But these are laboratory results. Does the tmema occur in nature? Is it adaptive? Could it permit small fragments of the protonema to have one more chance at dispersal before producing its upright gametophore, hence possibly allowing it to arrive at a place where it could indeed produce enough of its own IAA in a more favorable setting? How remarkable a survival mechanism if indeed it permits another chance at dispersal.

Tmema are one means of providing vegetative reproductive structures on the protonema. Various protonematal asexual reproductive structures will be

discussed in a later development chapter on asexual reproduction.

## Summary

The **filamentous protonema** of Bryophyta can differentiate into two types: **chloronema** and **caulonema**, distinguished by short cells with perpendicular crosswalls, numerous chloroplasts, colorless cell walls, and irregular branching in the former and longer cells, diagonal crosswalls, brownish cell walls, and fewer, scattered, small chloroplasts in the latter. IAA induces the switch to caulonema; cytokinins promote branching. Protonemata of Sphagnopsida, Anthocerotophyta, and most Marchantiophyta are thalloid.

Protonemata can produce a variety of **brood cells**, possibly stimulated by **ABA**, and sometimes disarticulated from the protonema by **tmema** cells. Light quantity, quality, photoperiod, and temperature influence both the rate of development and the form of the protonema. Their direction of growth is influenced by both gravity and light, causing **negative gravitropism** in the dark and **positive phototropism** in the light.

Other organisms may supply **IAA** and **GA** that influence development, and **Factor H** (a likely **cytokinin**) may be supplied both endogenously and exogenously to control population size. Nutrients can affect the development, with heavy metals generally causing abnormalities or arrested development.

**Rhizoids** exhibit **positive gravitropism** and **negative phototropism**, but also possess **thigmotropism**, typically expanding, branching, or flattening upon contact with a substrate.

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