THE USE OF MOSSES FOR THE STUDY OF CELL POLARITY

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Abstract. Moss development allows both the study of the development of cell polarity and the modification of polarity in an already-polar cell. The gametophore allows polarity studies to be extended to multicellular structures. Both spore germination and protoplast regeneration allow the study of the generation of a polar cell axis. The polarity of the axis of regenerating protoplasts of Ceratodon purpureus is influenced by light direction. The programming of the response is however complex. There is a delay before a response to a changed light direction is observed, indicating that axis polarity is fixed before asymmetrical development can be observed. However, the length of the delay is influenced by the state of the cell at the time the light direction is changed. When protoplasts regenerating in red light at 25°C are reoriented with respect to the light direction, there is a lag of about 9 hours before a response is observed. If protoplasts are irradiated with far-red light immediately before reorientation, the lag is shorter, indicating that protoplasts use phytochrome to "memorize" light direction, preventing a precipitous response to temporarily changed conditions. Protonema apical cells show tropic responses to both light and gravity. Mutant studies show that the phototropic response is mediated by phytochrome, and that this photoreceptor also turns off the gravitropic response if light. Mutants with a reversal of the orientation of their gravitropic response, have been isolated in both Physcomitrella patens and C. purpureus, and it is possible that in the latter species, a single gene can mutate either to prevent the gravitropic response or to reverse its orientation.

1. INTRODUCTION

We have defined polarity, in the context of developmental biology, as "the persistent, asymmetrical and ordered distribution of structures along an axis" (Cove et al., 1999). Moss development provides unrivalled opportunities to study polarity, not only at the level of the individual cell but also in multicellular organs. Both spore germinating spores and regenerating protoplasts provide material for the study of the acquisition of a polar axis by an apparently-unpolarised cell. The responses of protonemal apical cells to light and gravity, provide opportunities for the elucidation of the mechanisms by which the polarity of a cell's pre-formed axis can be modified in response to changed environmental inputs. The phototropic and gravitropic responses of gametophore allow similar studies to be made on a multicellular organ. The formation of three-dimensional buds from two-dimensional filaments allows the study...
of asymmetrical cell division during the transition from a two to a three dimensional structure. Furthermore, the haploidy of the gametophyte generation allows the direct isolation of mutants altered in aspects of cell and organ polarity, and simplifies their subsequent genetic analysis.

![Images of spores and regeneration](Image)

Figure 1 a. Germinating $P. \text{patens}$ spore, scale bar 50 $\mu$m; b. $C. \text{purpureus}$ regenerating protoplast, scale bar 50 $\mu$m; c. d. Distribution of axes of protoplasts of $C. \text{purpureus}$ regenerating in monochromatic c. red light (665 nm) and d. blue light (437 nm) (Cove et al, 1996)

2. SPORE GERMINATION

Moss spores germinate by polar outgrowth, to form protonemal filaments that extend by tip growth of the apical cell and its serial (figure 1 a). The induction of spore germination in $Physcomitrella \text{patens}$ requires light (Cove et al., 1978) but this does not hold for all moss species. Spores of $Fimaria \text{hygrometrica}$ germinate in darkness (Bauer and Mohr, 1959) but the point of emergence of the protonemal filament is sensitive to light (Jaffe and Etzold, 1965). In low intensities of red light, filament apices are produced towards the direction of the light source, whereas at high light levels, filaments emerge from the shaded side of the spore. The position that protonema emerge from the spore coat in $P. \text{patens}$ is not however cued by the direction of light (Schild, 1981) so it appears that in this species, light does not play a role in determining the orientation of the polar axis formed during spore germination. Gravity also appears to play no role (Schild and Cove, unpublished data).

3. PROTOPLAST REGENERATION

Isolated protoplasts from a number of moss species have been shown to regenerate by polar outgrowth, resembling germinating spores (see figure 1 b). In
Ceratodon purpureus there is clear evidence that light plays a role in setting up the polar axis (Cove et al. 1996). By observing the polarity of the regeneration axes of populations of protoplast cultured in unidirectional monochromatic light, it has been established that in red light, axes are well aligned with the light direction, but only 65-75% of protoplasts show polar outgrowth towards the light source, the remainder growing away from it. In blue light, alignment with the direction of the light source is poorer but almost all axes are oriented towards the light source. Figure 1c and d show circular histograms of the polarity of regeneration axes for these two light conditions.

Protoplast regeneration is not synchronous. At 25°, some protoplasts become asymmetrical as early as 15h after isolation, but the slowest protoplasts do not regenerate until 50 h after isolation. The time at which regeneration occurs in protoplasts left undisturbed in a unilateral light source, does not affect the alignment nor the orientation of the axis. Samples of protoplasts regenerating early or late show the same distribution of axis polarity. When protoplasts are re-oriented with respect to light direction during the course of regeneration, there is a lag of about nine hours (at 25° in monochromatic red light) before regeneration axes become aligned to the new light direction. As a result, it has been proposed (Cove et al, 1996) that the regeneration axis is fixed sometime before protoplasts become visibly asymmetrical. Reorientation affects not only axis alignment, but also whether outgrowth occurs towards or away from the light source (axis orientation). Soon after the light direction is changed, the proportion of protoplast orienting towards the light source declines. Although axes become aligned to the new light direction after a lag, differential orientation towards the light source does not occur, and equal numbers of protoplasts orient away and towards the light source. As a result of this, it has been proposed (Cove et al, 1996) that axis alignment and axis orientation may require different signal-transduction pathways.

Recent work (D.J. Cove, E. Hartmann, T. Lamparter, and R.S. Quatrano, unpublished) has investigated the role of phytochrome in determining the polarity of the protoplast regeneration axis. Samples of protoplasts which had started to regenerate in red light, were treated with far-red light, before being re-oriented and exposed to red light from a new direction. The far-red treatment results in the "memory" of the first light direction being lost more quickly, and a more rapid response to the new light direction, compared to the control (no far-red) treatment. However, if far-red light treated protoplasts are briefly returned to red light from the original direction before reorientation, the protoplasts' normal regeneration program is restored, i.e., protoplasts treated in this way resemble control protoplasts. These experiments provide evidence that phytochrome is involved in the perception of light direction. We further propose that the morphogen gradient responsible for the establishment of the polarity of the protoplast regeneration axis, must be stabilised by phytochrome in its Pr form, resulting in the response to a new light direction being slow, but that the gradient is less stable when phytochrome is in its Pr form, allowing a response to a new light direction to occur more rapidly.
4. **CHLORONEMAL APICAL CELL PHOTOTROPISM**

In *P. patens*, primary chloronemata, *i.e.* the protonemal filaments that emerge from germinating spores, show a polar response to both unidirectional and polarised light (Jenkins & Cove, 1983a). The response is sensitive to both the wavelength and intensity of the light. In high intensities of red light, the polarotropic response of apical cells is to align their growth axis parallel to the electrical vector of the plane polarised light. The corresponding phototropic response is alignment perpendicular to the direction of the light source ("high intensity" response).

In low intensities of polarised red light, growth is aligned perpendicular to the E vector, while in low intensities of unidirectional light, the phototropic response is alignment parallel to the light direction, with most filaments growing towards the light source, but a few growing away ("low level" response). In red light, the change from the low to the high level response occurs in the range of 1 – 3 μmol quanta m\(^{-2}\) s\(^{-1}\). At all wavelengths tested (range 417 to 730), both a polarotropic and a phototropic response occurred. In wavelengths between 540 and 600 nm and above 700 nm only a low level response was observed even at the highest fluence rates available. In wavelengths below 440 nm, only the high intensity response was observed even at the lowest fluence rates at which growth occurred. However, higher irradiances of blue light tended to elicit growth away from the light source rather than the observed response to high irradiances of red light, of growth perpendicular to the light source. In other wavelengths, a transition from the low level to the high level response was observed, but not necessarily at the same fluence rate. Table 1 summarises these results.

These same studies (Jenkins & Cove, 1983a) also examined the possible role of phytochrome in the phototropic and polarotropic responses. When far red light (730 nm) was shone from above onto a Petri dish irradiated through the edge with a high intensity of red light (665 nm), the response to red light was similar to that observed for a much lower fluence rate of red light (*i.e.* in the absence of the far red irradiation). Thus phytochrome appears to be involved at least in the detection of the light intensity at which the switch from a low level to a high level response occurs.

A parallel study (Jenkins & Cove, 1983b), examined the phototropic and polarotropic responses of mutants that had been isolated as a result of their being deficient in the phototropic response of their caulonemal apical cells and their gametophores (see below). It was found that the primary chloronemal apical cells of these mutants still showed both phototropic and polarotropic responses. However, the responses differed from those of the wild type. Surprisingly, the mutants switched from the low level to the high level response at lower fluence rates. For example, in red light (665 nm), mutants showed a high level polarotropic response even in the lowest fluence rates tested (300 nmol quanta m\(^{-2}\) s\(^{-1}\)). In green light (542 nm), the wild type showed a low level response at all intensities tested (maximum 70 μmol quanta m\(^{-2}\) s\(^{-1}\)), while the mutants switched for a low level to a high level phototropic response at fluence rates around 3 μmol quanta m\(^{-2}\) s\(^{-1}\).
Table 1. Relationship between fluence rate and polarotropic response of primary chloronemal apical cells of *P. patens*. (intensity = inferred fluorescence rate at which filaments change their alignment from parallel to perpendicular to the E vector of plane polarised light).

<table>
<thead>
<tr>
<th>wavelength (nm)</th>
<th>intensity (µmol quanta m⁻² s⁻¹)</th>
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<tbody>
<tr>
<td>417</td>
<td>&lt;1</td>
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<tr>
<td>442</td>
<td>0.5</td>
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<tr>
<td>473</td>
<td>1</td>
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<td>498</td>
<td>3</td>
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<tr>
<td>542</td>
<td>&gt;50</td>
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<tr>
<td>578</td>
<td>&gt;50</td>
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<td>613</td>
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<td>637</td>
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<td>665</td>
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<td>687</td>
<td>1</td>
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<tr>
<td>715</td>
<td>&gt;50</td>
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<td>730</td>
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Nebel (1968) obtained an action spectrum for the phototropic response of chloronemal apical cells of *Physcomitrium turbinatum*. He obtained a peak response with wavelengths around 730 nm, the absorbance peak for phytochrome in its P<sub>FR</sub> form.

5. CAULONEMAL APICAL CELL PHOTOTROPISM.

In *P. patens*, extension of caulonemal filaments occurs in a similar way to that of chloronemal filaments, by the apical extension of the filament apical cell. Caulonemal apical cells extend 10 – 15 times more rapidly than chloronemal apical cells, and also differ from them, in growing in darkness. Caulonemal filaments are therefore able to function as the adventitious phase of protonemal growth, and allow the rapid colonisation of the soil surface. The polarity of extension of the caulonemal apical cell is sensitive to both light and gravity. In low intensities of red light, apical cell extension occurs towards the light source, but at high intensities, growth is perpendicular (Cove and Lamparter, 1998). No detailed studies on the phototropic response of caulonemal apical cells in this species have been published, but preliminary experiments (Cove et al, 1978) indicated that phytochrome was involved in light perception. In a 23 h darkness, 1 h red light cycle, caulonemal apical cells show a phototropic response. However, when grown in a cycle comprising 22.75 h darkness, 1 h red light, 0.25 h far red light, no phototropic response was observed and
caulonemal apical cells instead showed the characteristic gravitropic response of upward growth, normally shown only in darkness.

It is not possible to differentiate morphologically between chloronemal and caulonemal filaments in the protonemata of *Ceratodon purpureus*. Protonemal apical cells of rapidly-growing axial filaments resemble caulonemal apical cells of *P. patens*, in responding to the direction of both light and gravity, but in contrast to *P. patens*, the phototropic response to red light, is towards the light source in both low and high light intensities. Extensive studies of the phototropic response have been carried out which demonstrate a clear involvement of phytochrome in this phototropic response (Hartmann et al, 1983). Filaments that have been growing in darkness, were exposed to light of different wavelengths and different intensities at right angles to the gravity vector. The angle of growth adopted by the filament was measured and dose response curve constructed. The resulting action spectrum for eliciting the positive phototropic response shows a clear peak at 660 nm. The phototropic response can be reversed if the red light treatment is immediately followed by exposure to far red light. The peak wavelength for far-red reversal is 730 nm. Protonemal apical cells of *C. purpureus* that have been grown in darkness and then illuminated from the side, show a rapid redistribution of the intra-cellular calcium gradient before any morphological change in cell polarity can be observed (Hartmann & Weber, 1988). The highest calcium concentration shifts from the cell apex to towards the light source. Following lateral illumination with red light, early re-organisation of the actin cytoskeleton in protonemal apical cells is also observed (Meske & Hartmann, 1995). This work also showed that anti-microtubule drugs (monensin and colchicines) did not inhibit the phototropic response, and so provided evidence that the microtubule cytoskeleton was not involved in phototropism. It therefore seems likely that calcium and the actin cytoskeleton are both involved downstream of phytochrome in mediating the adjustment of cell polarity to light direction.

Experiments employing the ionophore, monensin, reveal a remarkable aspect of the response of protonemal apical cells to lateral illumination (Hartmann & Weber, 1988). Monensin inhibits polar extension, but can be washed out, allowing growth to resume. If a filament that has been growing in darkness, is treated with monensin and then irradiated laterally with red light, no response can occur, because growth is inhibited. If the irradiation is stopped and the monensin is then washed out, a response to the direction of irradiation occurs. The apical cell can therefore retain a memory of the light direction, even if several hours elapse between the termination of irradiation and the washing out of monensin. The response to red light remains far-red reversible, providing evidence that the phytochrome itself, may be involved in the memory process. However, apical cells that are growing in the absence of monensin do not show the same memory of the light input and need to be irradiated continuously to elicit a phototropic response.

Mutants, impaired in their phototropic response, have been isolated in both *P. patens* (Cove et al, 1978) and *C. purpureus* (Lamparter et al, 1996). Aphototropic mutants of *P. patens* involve at least three complementary genes. Mutations in *pirA* and *pirB* lead to the pleiotropic loss of the phototropic response of both caulonemal
showing negative gravitropism) (see figure 2).

Strong growth of filaments in darkness is only obtained on media supplemented with either glucose or sucrose. Apical extension of caulonemal filaments of *P. patens* occurs at about 30 - 40 μm/h at 25° (Knight and Cove, 1991). Growth of *C. purpureus* filaments in darkness is more vigorous, and apical extension occurs at about the same rate for wild type 3, but somewhat faster for wild type 4 (see figure 2). The gravitropic response is inhibited by exposure to light, even at low

Figure 2. Negatively-gravitropic growth of protonemata in darkness.

a. *P. patens* Gransden Wild Type (Ashton & Cove, 1977) grown on minimal medium supplemented with sucrose. After initial culture for 14d in white light, the Petri dish was placed vertically and transferred to darkness for a further 21d.

b. *C. purpureus* Wild Type 3 (Hartman et al, 1983) cultured as in a, but transferred to darkness for 14d after incubation in light for 3d.


Scale bar for = 10 mm

intensities (see below). When dark-grown caulonemal filaments of *P. patens* are re-oriented by 90° with respect to the gravity vector, a 90° response gravitropic is achieved in about 72 h (see figure 3). Protonemal filaments of *C. purpureus* respond more rapidly, a full response to 90° reorientation being achieved in about 24 h (Walker and Sack, 1990). Time lapse video microscopy of filaments responding to reorientation to the gravity vector, is possible by filming in long wavelength light (>850 nm) and using an infra-red sensitive camera (Knight and Cove, 1991, Young and Sack, 1992, Schwuchow et al, 1995).

These studies reveal that the negative gravitropic response is not continuous. Immediately following reorientation, growth is towards the gravity vector rather than away and positively gravitropic growth also occurs during each mitotic division of the apical cell. These are not the only circumstances under which a positively gravitropic
response of a wild-type apical cell is observed. A positive response occurs in *P. patens* filaments, immediately upon transfer to a clinostat (Knight and Cove, 1991) and

![Figure 3. Growth response of a caulonemal filament of *P. patens* to 90° reorientation with respect to the gravity vector. Scale bar = 400 μm](image)

in *C. purpureus* filaments, when grown in monochromatic blue light (Lamparter *et al.*, 1998b). The significance of these positively-gravitropic response is not understood, but it has been suggested that the partial disassembly of the microtubular cytoskeleton during mitoses, may interfere with the response to gravity at those times (Knight and Cove 1991). Evidence for the role of the microtubular cytoskeleton in gravitropism is provided by inhibitor studies (Schwuchow *et al.*, 1990; Meske and Hartmann, 1995). Whereas the anti-microtubule drug, oryzalin does not inhibit the phototropic response of *C. purpureus* protonemal apical cells, this drug inhibits the gravitropic response.

There is a marked zonation of plastids along the long axis of apical cells of protonemal filaments of *C. purpureus* (Walker & Sack, 1990) and of caulonemal filaments *P. patens* (Knight & Cove, 1991) and *F. hygrometrica* (Schwuchow *et al.*, 1995). In *C. purpureus*, there is a zone of plastids at the immediate tip, which is absent in the other two species. In all three species, moving away from the cell apex, there is a plastid-free zone. Next there is a zone of amyloplasts which exhibit clear gravity-stimulated sedimentation in *C. purpureus* (Walker & Sack, 1990) and *F. hygrometrica* (Schwuchow *et al.*, 1995), and finally a zone of containing non-sedimenting plastids and a vacuole. Plastid sedimentation in *P. patens* is not obvious (Knight & Cove, 1991) but some movement is detectable (Schwuchow *et al.*, 1995). It is attractive to propose that the sedimenting zone of amyloplasts is the site of gravity perception by apical
cells, but studies of regenerating protoplasts reveal that emerging filaments become graviperceptive before plastid zonation or sedimentation can be observed (Wagner & Sack, 1998).

In a preliminary study of the interaction between phototropism and gravitropism in *P. patens* (Cove et al., 1978), protonemal growth in light was found to be “completely indifferent to gravity”. Jenkins et al. (1986) found that in intensities of monochromatic red light above 200 nmol quanta m⁻² s⁻¹ no sensitivity to gravity could be detected. When irradiated at right angles to the gravity vector, with monochromatic red light at an intensity of 90 nmol quanta m⁻² s⁻¹, caulonemal apical cells appear to make a choice between positive phototropic and negative gravitropic, resulting in filaments that switch from time to time, between upwards growth and growth towards the light, but irradiation at an intensity of 60 nmol quanta m⁻² s⁻¹, results in growth at 45° to both the light direction and gravity (Cove & Knight, 1987, Jenkins & Cove, unpublished data). These studies suggest that there is competition between gravitropism and phototropism, and that phototropism is the stronger morphogenetic input. However, studies of aphototropic mutants of both *P. patens* and *C. purpureus* show that the interaction of gravitropism with light, is not simply competitive. Thus aphototropic mutants of *P. patens*, which have a wild-type gravitropic response in darkness, do not respond to gravity in light levels above 1 μmol quanta m⁻² s⁻¹ (Cove & Knight, 1987). Mutants of the second class of aphototropic mutant of *C. purpureus*, (impaired downstream of phytochrome - see above), show a similar lack of gravitropism in light. On the other hand, *C. purpureus* mutants that are impaired in the synthesis of the phytochrome chromophore, continue to respond to gravity even in high light levels (Lamparter et al., 1996), providing strong evidence that gravitropism is actively switched off in the light, by way of phytochrome.

Mutants showing an abnormal response to gravity (*gtr* mutants), have been identified in both *P. patens* and *C. purpureus*. Genetic analysis of *P. patens* *gtr* mutants has been carried out using somatic hybridisation (Jenkins et al., 1986). This initial study revealed that at least three genes are involved. *gtrA* mutants are impaired in their response to gravity but even those most extremely affected, still show a tendency to grow upwards. These mutants show a pleiotropic effect on gametophore development, having more rounded leaf cells and consequently more fleshy leaves. Mutants isolated initially as a result of their having this leaf morphology, have been shown subsequently to be impaired in their gravitropic response, confirming this pleiotropy. A second gene was identified by a single mutant, *gtrB1*. The morphology of the caulonemal apical cells of this mutant was abnormal with no plastid zonation observable (Jenkins et al., 1986). Unfortunately, this mutant was later lost and no similar mutants have since been reported. A third gene was identified in the original study, again by a single mutant, *gtrC5*. This mutant produced caulonemal apical cells that responded positively to gravity, *i.e.* grew downwards. A subsequent more extensive genetic analysis (Knight, 1987) included more mutants. This identified a further gene, also represented by a single mutant, *gtrD8*, which like *gtrA* mutants showed a pleiotropic effect on leaf morphology. This study included more *gtrC* alleles, all with caulonemal apical cells showing a positive gravitropic response. Genetic analysis showed that the *gtrC7*
mutant segregated in a regular manner, confirming that this phenotype arises as a result of mutation in a single gene.

All mutants of *P. patens* showing an abnormal gravitropic response of the caulonemal apical cells, produced gametophores showing the wild-type negatively gravitropic response, contrasting with mutants affected in phototropism, where the response of both caulonemal apical cells and of gametophores was affected (see above). No mutants having gametophores impaired in their response to gravity have been reported.

Mutants of *C. purpureus* with an altered protonemal response to gravity are readily selected as a by-product of the isolation of phototropically-abnormal strains (Lamparter *et al.*, 1996; Wagner *et al.*, 1997). Mutants resembling *P. patens* gtrA in showing a weak or very weak negatively gravitropic response appear to be less common (Cove, unpublished data), but mutants responding positively to gravity are more easily identified. The first of these to be examined physiologically was designated wwr1 (for wrong way response), although subsequent genetic analysis suggests that a similar mutant may be an allele of a gene that can mutate to give a near-agravitropic phenotype (see below). The wwr1 mutant was found to have a gravitropic response which mirrored that of the wild type, in that initially and during nuclear division it grew upwards, and at other times down (Wagner *et al.*, 1997). The protonemal apical cells of the wwr1 mutant show similar but not identical plastid zonation to the wild type, and plastid sedimentation is obvious in the mutant.

The absence of mutants in both *P. patens* and *C. purpureus* that are completely agravitropic, suggests that there may be some overlap of gene function such that knock out of any one gene does not abolish sensitivity to the gravity vector completely. The recent report that *C. purpureus*, grown in microgravity, has a characteristic symmetrical spiral growth (F. Sack unpublished data, see [http://spaceresearch.nasa.gov/general_info/16jul_firemoss_lite.html](http://spaceresearch.nasa.gov/general_info/16jul_firemoss_lite.html)), suggest that this is the predicted phenotype of a mutant strain completely insensitive to gravity and a further mutant hunt beginning with a near-agravitropic strain, might identify a further gene involved in gravisensing.

Genetic analysis of gravitropic mutants of *C. purpureus*, is in progress (Cove, unpublished data). Somatic hybrids are selected using transgenic antibiotic resistant strains obtained following transformation. Hybrids may be selected between strains having resistances to different antibiotics, by counter-selecting for both antibiotics. Figure 4 shows a series of hybrids obtained in this manner. The control (homozygous) hybrids, show the phenotypes of the wild type and gravitropic mutants involved, one of which is almost completely impaired in its response (gtr406), and the other (gtr445) showing a reversed positively-gravitropic response.

The hybrids between gtr406 and wild type, and between gtr445 and wild type, both show the wild-type response, confirming that both mutant phenotypes are recessive. The gtr406/gtr445 does not however show a wild-type response as would have been predicted had the two phenotypes been the result of mutation in different genes. The phenotype of the gtr405/gtr446 hybrid may indicate that a single gene can mutate to give rise to alleles which result in either loss or reversal of gravitropism, in
which case the positively-gravitropic phenotype presumably involves only a partial loss of gene function. Alternatively, *gir405* may be a double mutant, agravitropism being epistatic to the reversed response. Analysis involving more mutant strains, which is currently in progress, may allow these possibilities to be distinguished.

Figure 4. Growth of somatic hybrids of *C. purpureus* in darkness.

a  wild type plus wild type  d  *gir406* plus wild type
b  *gir406* plus *gir406*  e  *gir445* plus wild type
c  *gir445* plus *gir445*  f  *gir406* plus *gir445*

Somatic hybrids were obtained by fusion of protoplasts from two transgenic strains containing genes conferring resistance to different antibiotics, following by selection for strains resistant to both antibiotics (Cove, unpublished data). Scale bar: 10 mm
7. GAMETOPHORE GRAVITROPISM AND PHOTOTROPISM

There are no reports of extensive studies on gametophore tropisms. In *P. patens*, the development of new gametophores does not occur in darkness, but gametophores present upon transfer to darkness, etiolate and grow upwards (Cove *et al.*, 1978). As with the response of caulonemal apical cells, even low levels of light inhibit the gravitropic response of gametophores, with those of the wild type growing toward the light and those of phototropic mutants oriented randomly. It therefore seems likely that gametophore gravitropism is also actively switched off by light.

8. FUTURE PROSPECTS

Studies to date have shown that moss development provides outstanding material for the study of polarity at both the level of the individual cell and in multicellular structures. To advance these studies, more detailed investigation of the cellular events involved in the establishment and modification of polarity, using modern cell biological techniques, need to be combined with molecular genetic studies of the genes involved. If progress is forthcoming, moss development has the potential to make landmark contributions to our understanding of biological polarity.

Public access to an extensive EST database, as well as libraries of genomic DNA, bacterial artificial chromosomes (BACs), and cDNAs, are now available for *P. patens* (see - www.moss.leeds.ac.uk). These resources provide the possibility of identifying genes of potential interest in relation to polarity, by their homology to genes identified in other systems, including higher plants (see for example Fu *et al.*, 2001), the products of which play a role in the establishment and/or maintenance of a polar axis. Coupling these resources with the ability to remove or replace specific genes and/or promoters by homologous recombination (Schaefer, 2001) provides a unique opportunity in plants to localize and to determine the function of specific gene products in cell polarity. The ability to target transgenes to specific genomic loci (Schaefer, 2001), the disruption of which does not have any noticeable effect on the development of *P. patens*, allows the opportunity to assess in stable lines of *P. patens*, the results of ectopic expression of genes of interest. Effects on cell polarity of the over-expression of genes, and the expression of anti-sense and/or RNAi constructs can be assessed, independent of the complexities of interpreting the effects of transgenes in different chromatin environments. Similarly, the replacement of wild-type genes with alleles of specific interest will allow an assessment of the effects of the allelic substitution itself which will not be subject to effects due to the chromosomal environment. This is not yet possible in *Arabidopsis thaliana*, where for example, Fu *et al.* (2001) have shown specific effects of At ROPI alleles on pollen tube growth, but due to the nature of transformation in *A. thaliana*, each of these alleles is located in a different chromosomal environment, not that of its wild type counterpart. A very similar homolog to the *A. thaliana* ROPI gene is present in the *P. patens* genome. The
tools now exist in *P. patens* to study the effects of these same allelic substitutions on filament tip growth at the same (wild-type) chromosomal location.

9. REFERENCES


