

# Agravitropic mutants of the moss *Ceratodon purpureus* do not complement mutants having a reversed gravitropic response

DAVID J. COVE<sup>1,2</sup> & RALPH S. QUATRANO<sup>2</sup>

<sup>1</sup>Centre for Plant Sciences, University of Leeds, Leeds LS2 9JT, UK and <sup>2</sup>Department of Biology, Washington University in St. Louis, St. Louis, MO 63130-4899, USA

## ABSTRACT

**New mutants of the moss *Ceratodon purpureus* have been isolated, which showed abnormal gravitropic responses. The apical cells of protonemal filaments of wild-type strains respond to gravity by growing upwards and are well aligned to the gravity vector. This response only occurs in darkness. Mutants show a range of phenotypes. Some are insensitive to gravity, showing symmetrical growth, while others align to the gravity vector but orient growth downwards. A further class grows in darkness as though it were in light, showing insensitivity to gravity and continued chlorophyll synthesis. Somatic hybrids between mutants and wild-type strains and between pairs of mutants have been selected using transgenic antibiotic resistance as selective markers. Hybrids between wild-type strains and all of the mutants have a wild-type phenotype, and so all mutants therefore have recessive phenotypes. Mutants comprise three complementation groups. One group has a single member, while another has three members. The third has at least 16 members and shows a complex pattern of complementation consistent with a single gene product functioning in both orientation and alignment to gravity, as well as contributing more than one subunit to the mature product.**

*Key-words:* axis alignment, cell polarity; *cop/det* mutants; gravitropism; interallelic complementation; somatic hybrids.

## INTRODUCTION

The apical cells of filaments of the protonemal stage of moss gametophyte development provide an excellent material for the study of cell polarity (Cove & Quatrano 2004). The perception of external stimuli, their transduction and the response all occur within the apical cell of the protonemal filament. The cell response can be observed directly in real time. The protonemal tissue is haploid, making mutant isolation and genetic analysis straightforward.

Protonemal filaments of *Physcomitrella patens* are differentiated into chloronemata and caulonemata (for review, see Cove 2005). The apical cells of the two filament types show different responses to light inputs and only caulonemal apical cells respond to gravity by growing upwards. The gravitropic response is inhibited by light (Cove *et al.* 1978). In cells that are reoriented 90° with respect to the gravity vector in darkness, the growth of the apical cell away from gravity is not continuous and is reversed both immediately after reorientation and during subsequent cell divisions (Knight & Cove 1991). The protonemal tissue of *Ceratodon purpureus* does not show distinct chloronemal and caulonemal filament differentiation, but mature filaments respond to gravity in darkness in a similar manner to caulonemata of *P. patens* (Walker & Sack 1990). A small number of mutants of *P. patens* showing abnormal responses to gravity have been isolated, and at least three genes have been shown to be involved (Jenkins, Courtice & Cove 1986). Mutations in one of these, *gtrC*, still align to gravity, but orient their growth downwards. Previously, only one *gtr* mutant of *C. purpureus* has been reported and this, like the *gtrC* mutants of *P. patens*, aligned to gravity but grew downwards. This mutant was named ‘wrong way response’ (*wwr*) (Wagner, Cove & Sack 1997).

Thus, in both *P. patens* and *C. purpureus*, the response of the wild-type to reorientation and a mutant phenotype suggest that the qualitative response to gravity, upward or downward growth, and the quantitative response, how closely growth is aligned to the gravity vector, are separable. Although the response of mosses to gravity provides a clear example of the uncoupling of the alignment and orientation of a polar response, it is likely that this does not represent an isolated phenomenon. Examples where reversal of the orientation of an axis occurs without altering its alignment are found in nematodes (Wood 1991), molluscs (Freeman & Lundelius 1982) and mammals (Layton 1976), but the moss system promises to be one of the most amenable for the experimental analysis of the relationship between axis alignment and orientation. Here, we report the isolation of a large number of new gravitropism mutants involving the loss of sensitivity to gravity and the reversal of the orientation of the response. These have been analysed using somatic hybridization to establish the genetic structure of gravitropism in *C. purpureus*.

*Correspondence:* David J. Cove. Fax: +44 113 343 3144; e-mail: d.j.cove@leeds.ac.uk

## MATERIALS AND METHODS

### Growth conditions

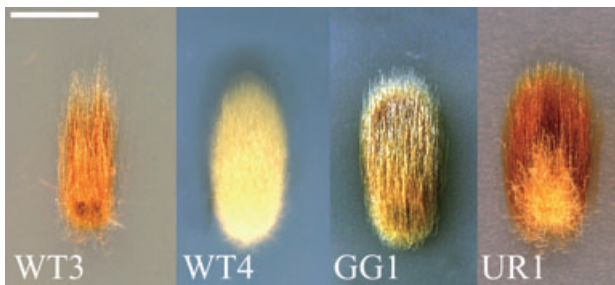
All tissue was grown at 25°. Cultures incubated in light were provided with continuous white light by fluorescent tubes at 50–80  $\mu\text{E}$ . Protoplasts were regenerated on a protoplast regeneration medium (PRM) (Knight *et al.* 2002). All other tissue was grown on BCD medium supplemented with 500 mM di-ammonium tartrate (BCD + A) (Knight *et al.* 2002), to which 50 mM sucrose was added for the culture in darkness. Antibiotics (Hygromycin and G418, Sigma, St. Louis, MS, USA) were added as indicated.

### Wild-type strains

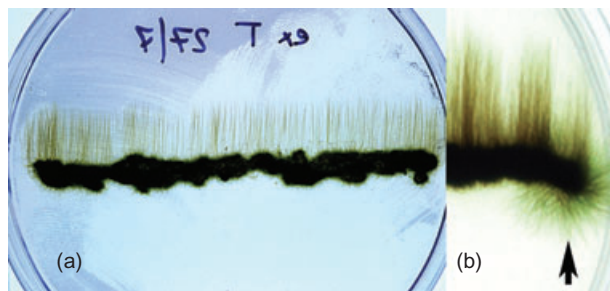
Four wild-type strains were used in these studies. The origins of wispertal 3 (WT3) and wispertal 4 (WT4) have been described previously (Hartmann, Klingenberg & Bauer 1983), the first wild-type isolated from Gross Gerungs (GG1) was obtained from a single spore from a capsule isolated at Gross Gerungs, Austria, by D.J.C. and the first ukrainian wild-type (UR1) was isolated by Dr Christina Chaban from a material collected in the Ukraine. The protonemata of all four strains respond to gravity in darkness by an upward growth (Fig. 1). The dark-grown protonemal filaments of WT3, GG1 and UR1 contain a brown pigment, whereas those of WT4 are unpigmented.

### Isolation of mutants with altered responses to gravity

With the exception of *gtr-301*, which was previously designated *wwr-1* (Wagner *et al.* 1997), all of the mutants used were isolated in this study. Mutants were isolated from WT3 (*gtr-300* series) following ultraviolet (UV) mutagenesis, from GG1 (*gtr-500* series) following either UV or ethyl methane sulphonate (EMS) mutagenesis, and spontaneous mutants were isolated from WT4 (*gtr-400* series).



**Figure 1.** Growth in darkness of the wild-type strains used in this investigation. The cultures were inoculated with clumps of protonemal tissue about 2 mm in diameter, onto a medium containing sucrose, incubated horizontally (i.e. placed flat on the incubator shelf) for 3 d in light, and then transferred to darkness and incubated vertically (i.e. placed on edge) for 14 d further. Scale bar = 10 mm. WT3, wispertal 3; WT4, wispertal 4; GG1, the first wild-type isolated from Gross Gerungs; UR1, the first Ukrainian wild-type.



**Figure 2.** Mutant screen. (a) Tissue that had developed from the regeneration of protoplasts that had been subjected to ultraviolet (UV) mutagenesis was scraped into strips that were transferred to a medium containing sucrose in 90-mm-diameter Petri dishes. The dishes were incubated horizontally for 3 d in light and then transferred to darkness and incubated vertically for 14 d further. The typical response of upward [negatively gravitropic] growth is evident. (b) Detail of the identification of a gravitropically abnormal regenerant (to the right).

### UV mutagenesis

Protoplasts were isolated from 7-day-old tissue and added to liquefied top layer PRM at 45° to give approximately  $5 \times 10^5$  viable protoplasts  $\text{mL}^{-1}$  (Cove, Quatrano & Hartmann 1996). The top layer PRM containing protoplasts was added to the PRM overlaid with cellophane at the rate of 2 mL per Petri dish and allowed to solidify. The dishes were then irradiated at 25° with a dose of  $1800 \text{ J m}^{-2}$  in a Stratagene UV Stratolinker 1800 (Stratagene, San Diego, CA, USA). After irradiation, the dishes were kept in darkness for 24 h at 25° and then incubated for 8 d further in light. About 2% of the treated protoplasts survived this treatment (about  $2 \times 10^4$  regenerants per dish). The regenerated tissue was then scraped with a sterile spatula into strips (three strips from each Petri dish of regenerants), and the strips were transferred to Petri dishes of BCD + A supplemented with 350 mM sucrose. These dishes were incubated in a horizontal orientation (i.e. placed flat on the incubator shelf) in light for 3 d and then oriented vertically (i.e. placed on the edge), transferred to darkness and incubated for 14 d further at 25°, after which mutant sectors could be identified (see Fig. 2).

### Mutagenesis with methane sulphonic acid ethyl ester (EMS)

EMS (250  $\mu\text{L}$ ) was added to approximately 1 g fresh weight (FW) of 7-day-old protonemal tissue, suspended in 10 mL of sterile 100 mM orthophosphate buffer (pH 7.0) and incubated 20 min at 25°. Ten millilitres of sterile 400 mM sodium thiosulphate solution was added to inactivate the EMS, the tissue harvested by collection on 100  $\mu\text{m}$  steel mesh and washed twice with 100 mL of sterile distilled water. The tissue was then resuspended in 10 mL of sterile distilled water, blended (Knight *et al.* 2002) and the resulting tissue suspension was distributed onto five dishes of BCD + A overlaid with cellophane and incubated for 7 d in light.

Thereafter, the procedure was similar to that for UV mutagenesis.

### Isolation of spontaneous mutants

Seven-day-old tissue growing on BCD + A overlaid with cellophane was scraped into strips (five to six strips from each Petri dish of tissue), and the strips of tissue were transferred to BCD + A supplemented with 350 mM sucrose. After 3 d incubation horizontally in light, the dishes were reoriented vertically and transferred to darkness for 14 d further.

### Production of transgenic strains

To introduce markers to allow the selection of somatic hybrids, strains were transformed using poly (ethylene glycol) (PEG)-induced uptake of DNA by isolated protoplasts, following the same procedure routinely used for the transformation of *P. patens* (Knight *et al.* 2002). G418 resistance was introduced by transformation with the pJIT161 plasmid and Hygromycin resistance with the pBI221-23 plasmid (Knight *et al.* 2002).

### Selection of somatic hybrids

The procedure used was based on that for the selection of somatic hybrids in *P. patens*, except that transgenic antibiotic resistances were used for the selection instead of vitamin auxotrophies (Grimsley, Ashton & Cove 1977). Protoplasts were isolated from the two strains to be hybridized, one of which carried a transgene for G418 resistance and the other a transgene for Hygromycin resistance. Approximately  $5 \times 10^6$  protoplasts of each strain were mixed and subjected to the fusion treatment. After washing, the protoplasts were resuspended in 1 mL 8% mannitol solution. The protoplast suspension (50  $\mu$ L) was added to 8 mL of molten top layer PRM, and the remainder of the suspension was added to a further 7 mL of top layer PRM. The top layer was then added at 2 mL per dish to PRM overlaid with cellophane. After 3 d of incubation in light, the cellophane overlays from the dilute platings were transferred to BCD + A containing either 50 mg L<sup>-1</sup> G418 or 30 mg L<sup>-1</sup> Hygromycin (two dishes each). The cellophane overlays from the concentrated platings were transferred to BCD + A containing both antibiotics. The platings on the media containing one or the other antibiotic were used to estimate the survival of the individual strains, and allowed the recovery of regenerants that served as a control. Somatic hybrids were identified by their growth on the medium containing both G418 and Hygromycin.

### Scoring gravitropic phenotypes

After selection, a sample of five hybrids from a hybridization was inoculated onto a Petri dish of BCD + A supplemented with 350 mM sucrose. In parallel, five regenerants from each of the single antibiotic plates were also inocu-

lated onto BCD + A with 350 mM sucrose. These dishes were incubated in continuous white light in a horizontal orientation for 3 d and then oriented vertically, transferred to darkness and incubated for 14 d further at 25°, after which gravitropic phenotypes were scored (see Fig. 3 for examples). After scoring, the cultures were transferred to light and incubated further. Under these conditions, the protonemata that had developed in darkness regenerated, and the tissue inocula were taken from these and tested for their antibiotic resistance phenotype. In all cases, the phenotype was as expected.

## RESULTS

### Mutant phenotypes

Table 1 lists the origins and gravitropic phenotypes of the mutants used in this study. Orientation is scored as up or down, but mutants which have little or no sensitivity to the gravity vector are scored as symmetrical. The phenotype of symmetrical mutants showed some variations; replica cultures of the same strain could show a slight upward bias or a slight downward bias, or could grow symmetrically. Alignment is scored on a scale of 1–4, where 1 is the least aligned and 4 corresponds to alignment to gravity similar to wild-type strains. Figure 3 gives examples of some of the phenotypes.

### Mutation rates

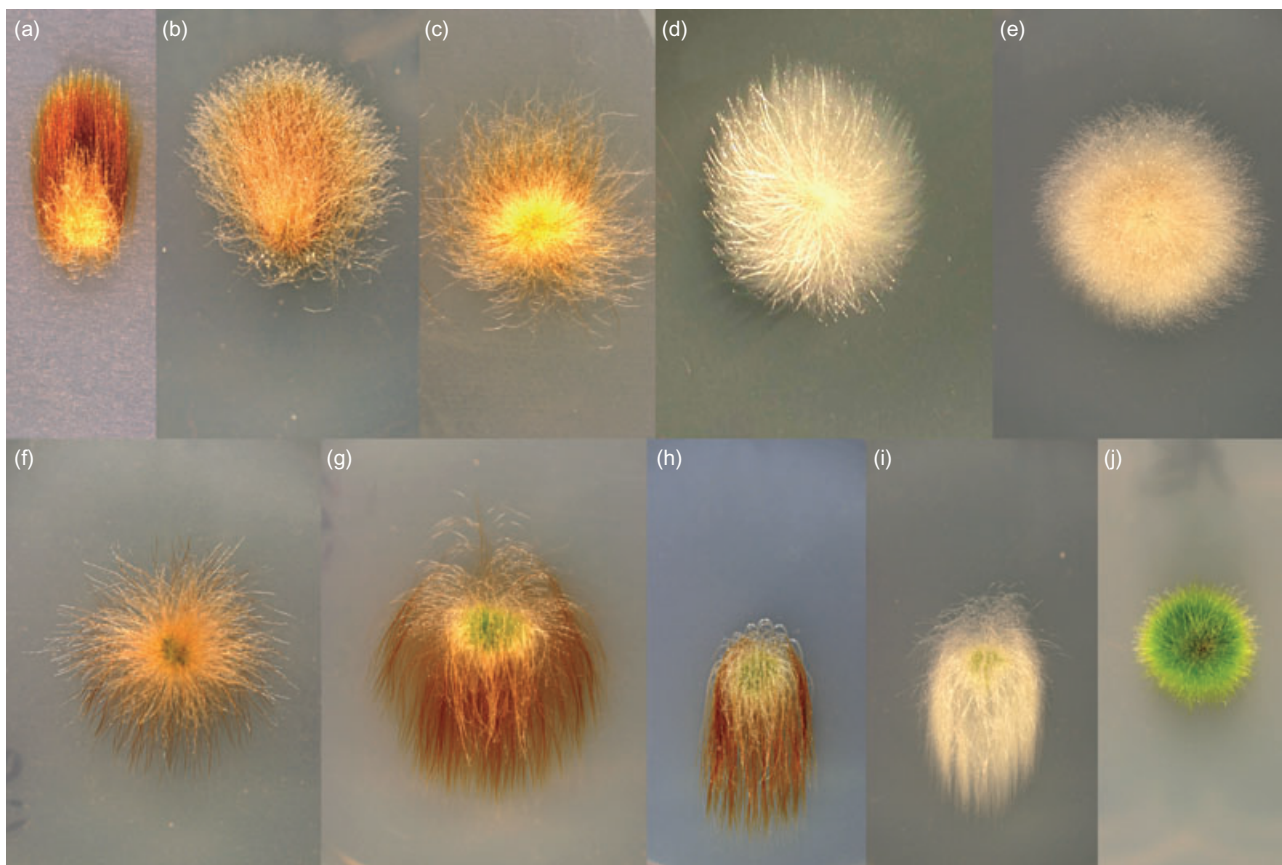
The three protocols used to obtain mutants all utilize strips of tissue and rely on self-identification of mutants (see Fig. 2b). It is not possible to quantify the rate of mutant generation, nor is it possible, because of the differences in the protocols, to compare the different methods quantitatively. No spontaneous *gtr* mutants were observed in any experiment involving WT3 or GG1, but WT4 gave rise to spontaneous mutants. WT4 therefore has a higher rate of spontaneous mutation. Notwithstanding this high rate of spontaneous mutation, most mutants obtained showed no subsequent instability, and only those that were stable were chosen for analysis. The basis of the mutational instability of WT4 is unknown.

### Hybridization rates

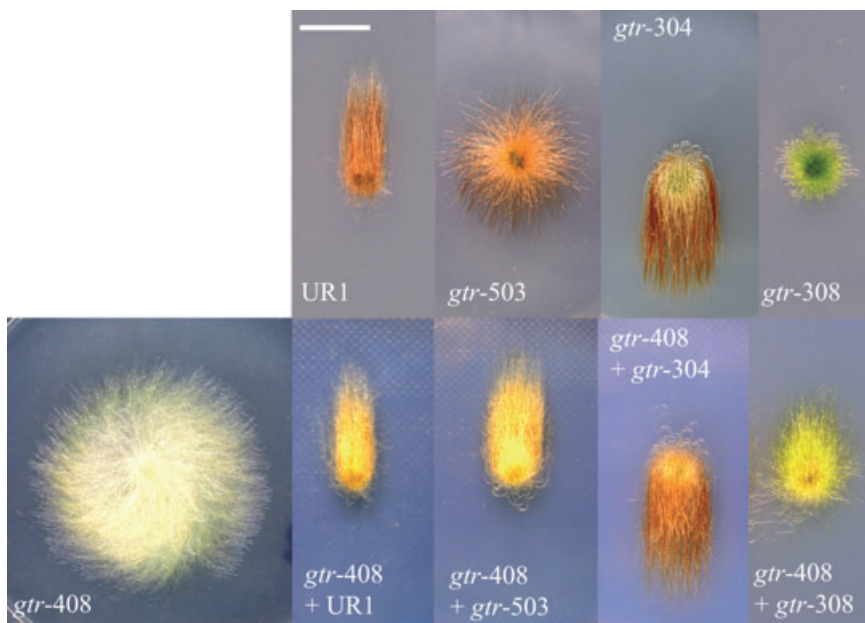
About 4% of the protoplasts survived the hybridization procedure. The frequency with which hybrids arose was variable, but it was usual to obtain about 20 hybrids per experiment (i.e. 1 hybrid per  $2 \times 10^4$  surviving protoplasts). A few pairs of strains failed to give rise to hybrids in several independent procedures, but other pairs generated over 100 hybrids in a single hybridization experiment.

### Hybrid phenotypes

Figure 4 shows examples of representative hybrid phenotypes, and Table 2 summarizes the phenotypes of parental



**Figure 3.** Examples of gravitropic phenotypes. (a) Gravitropism score: U4 (orientation: up, alignment: 4 [UR1 wild-type strain]). (b) Score: U3 (*gtr-301*). (c) Score: U2 (hybrid *gtr-406 + gtr-506*). (d) Score: U1 (hybrid *gtr-406 + gtr-415*). (e) Score: S (*gtr-421*). (f) Score: D1 (orientation: down, alignment: 1 (*gtr-503*)). (g) Score: D3 (*gtr-303*). (h) Score: D4 (*gtr-502*). (i) Score: D4 (*gtr-414*). (j) Score: S (*gtr-308*) *gtr-414* and *-421* were derived from the wispertal 4 (WT4) strain which lacked pigment as does the *gtr-406 + gtr-415* hybrid, both component strains of which were derived from WT4. The *gtr-406 + gtr-506* hybrid is pigmented similar to its *gtr-506* component which was derived from the pigmented GG1 wild-type. Pigment production can therefore be seen to be dominant. U, upward growth; D, downward growth; S, symmetrical growth.



**Figure 4.** Phenotypes of representative strains and hybrids. The growth conditions were as described in Fig. 1. Strain *gtr-408* is insensitive to gravity. Its hybrid with the UR1 wild-type strain shows a response similar to UR1 itself, and so *gtr-408* is recessive. Its hybrid with *gtr-503*, another mutant showing reduced sensitivity to gravity, also has a wild-type phenotype. *gtr-408* and *gtr-503* therefore complement, and so are impaired in different genetic functions. The hybrid between *gtr-408* and *gtr-304* resembles *gtr-304*, and so these two mutant strains are unable to complement to give a wild-type phenotype, and therefore must have overlapping functions. The hybrid between *gtr-408* and *gtr-308* grows upwards. The production of a brown pigment can be seen to be dominant to a lack of pigment, but the production of chlorophyll in darkness by *gtr-308* is at least partially dominant.

**Table 1.** Origin and phenotype of gravitropic mutants used in this study

Allele number	Group	Mutagenesis	Pigment	Orientation to gravity	Alignment to gravity
301	1	UV	Brown	Down	4
303	1	UV	Brown	Down	4
304	1	UV	Brown	Down	3
308	3	UV	Green	Symmetrical	–
310	3	UV	Green	Symmetrical	–
311	3	UV	Green	Symmetrical	–
403	1	Spontaneous	None	Up	2
406	1	Spontaneous	None	Symmetrical	–
408	1	Spontaneous	None	Symmetrical	–
410	1	Spontaneous	None	Down	4
414	1	Spontaneous	None	Down	4
415	1	Spontaneous	None	Up	2
421	1	Spontaneous	None	Symmetrical	–
428	1	Spontaneous	None	Down	4
444	1	Spontaneous	None	Symmetrical	–
445	1	Spontaneous	None	Down	4
446	1	Spontaneous	None	Down	3
502	1	UV	Brown	Down	4
503	4	EMS	Brown	Down	1
505	1	UV	Brown	Down	4
506	2	UV	Brown	Up	2
507	1	UV	Brown	Down	4

Two aspects of the gravitropic response are given. The orientation indicates whether protonemal growth in darkness is upwards or downwards, while alignment reflects how closely filaments are aligned to the gravity vector whether growing upwards or downwards, 4 being as aligned as the wild-type (see Fig. 3).

Except for *gtr-301* (Wagner *et al.* 1997), all mutants were newly isolated.

UV, ultraviolet; EMS, ethyl methane sulphionate.

strains and of all hybrids obtained in this investigation. Not all hybridizations were attempted, and only where 'no hybrids' is noted were attempts to select hybrids unsuccessful.

## DISCUSSION

### Somatic hybridization as a method of complementation analysis

Diploid moss hybrids produced by either aposporous regeneration of moss sporophytic tissues (Engel 1968) or somatic hybridization (Grimsley *et al.* 1977), developmentally resemble the haploid gametophyte generation. The gravitropic phenotype of the protonemal apical cells of somatic hybrids can therefore be used to determine the dominance status of mutant phenotypes, by selecting hybrids between mutant and wild-type strains, as well as for testing for complementation between mutant strains. All mutant strains included in this analysis, except *gtr-308*, -310 and -311, had recessive phenotypes, indicating that they were likely to be loss-of-function mutants. The phenotypes of hybrids between *gtr-308*, -310 and -311 and wild-type strains grew upwards, but continued to synthesize some chlorophyll in darkness.

Because all the gravitropic phenotypes of the mutant strains are recessive, a complementation analysis between mutant strains can determine whether the mutations have affected the same or different functions. Somatic hybridiza-

tion using transgenic selection markers also allows homozygous hybrids to be selected. As controls, a number of such homozygous hybrids were selected (see Table 2). In all cases, the phenotype of the homozygous hybrid was the same as the haploid strain. No Mendelian genetic analysis has yet been undertaken on these mutant strains. There are a number of reasons for this, not least that WT3 and WT4 appear to be sexually sterile probably as a result of prolonged vegetative subculture. Nevertheless, attempts are in hand to outcross these mutants, but to date somatic hybridization has been the only practical way available for genetic analysis.

### Complementation analysis

#### *gtr-503* (Poorly aligned to gravity)

The phenotypes of hybrids between mutant strains are summarized in Table 2. Mutant *gtr-503*, which has a near agravitropic phenotype (score: D1, see Fig. 3), complements all other strains (see e.g. Fig. 4) indicating that it is impaired in a function distinct from all other mutants.

#### *gtr-308*, -310 and -311 (Poorly aligned and constitutively photomorphogenetic)

These mutants all have similar phenotypes. In light, their growth is similar to that of the wild-type. In darkness, they are insensitive to gravity, and, unlike the wild-type or other

**Table 2.** The gravitropic phenotypes of hybrid and component strains

G418 <sup>r</sup> strain	Comp. group	Phenotype	Hygromycin-resistant strain															
			UR1	WT3	WT4	<i>grt</i> -301	<i>grt</i> -303	<i>grt</i> -304	<i>grt</i> -406	<i>grt</i> -408	<i>grt</i> -415	<i>grt</i> -428	<i>grt</i> -444	<i>grt</i> -446	<i>grt</i> -502	<i>grt</i> -506	<i>grt</i> -308	<i>grt</i> -113
UR1	-	U4	U4	U4	U4	1	1	1	1	1	1	1	1	1	1	3	S	3
WT3	-	U4	U4	U4	U4	U4	U4	U4	U4	U4	U4	U4	U4	U4	U4			
WT4	-	U4	U4	U4	U4	U4	U4	U4	U4	U4	U4	U4	U4	U4	U4			
<i>grt</i> -301	1	D4			D4													
<i>grt</i> -303	1	D3			D4													
<i>grt</i> -304	1	D4			D4													
<i>grt</i> -403	1	U2			D4				No hybs.	U2								
<i>grt</i> -406	1	S			D4				D1	U1								
<i>grt</i> -408	1	S			D4				D4	D4								
<i>grt</i> -410	1	D4			D4													
<i>grt</i> -414	1	D4			U4					D4								
<i>grt</i> -415	1	U2			U4					U1								
<i>grt</i> -421	1	S			U4					D4								
<i>grt</i> -428	1	D4																
<i>grt</i> -445	1	D4																
<i>grt</i> -446	1	D3			U4													
<i>grt</i> -502	1	D4			U4													
<i>grt</i> -505	1	D4			U4													
<i>grt</i> -507	1	D4			U4													
<i>grt</i> -503	2	D1			U4					U4								
<i>grt</i> -310	3	S			U3					U4								

Hybrids were selected by the fusion of protoplasts from two strains, one of which was Hygromycin resistant (strains listed across the top of the table) and the other resistant to G418 (strains listed down the left-hand column). The phenotype of a hybrid is given in the column and row relating to the two component strains. Phenotypes are coded as follows: U, upward growth; D, downward growth; 4, most aligned to gravity vector; 1, least aligned; S, symmetrical growth. For clarity, cells are coloured green for aligned upward growth, brown for aligned downward growth and blue for lack of or poor alignment. Heavily outlined cells are hybrids produced between hygromycin-resistant and G418-resistant derivatives of the same strain. Cells in which 'no hybs.' is noted are the only cases where attempts to select the relevant hybrid were unsuccessful. The row and column designated 'comp. group' indicate the complementation group to which each mutant strain is likely to belong. For further details, see the discussion section. UR1, WT3 and WT4; transgenic strains derived respectively from the Ukraine 1, wisperal 3 and wisperal 4 wild-type strains.

*gtr* mutant strains, they synthesize chlorophyll (see Fig. 3j). The gravitropic response in wild-type strains only occurs in darkness. Because mutants blocked in the synthesis of the phytochrome chromophore continue to respond to gravity in light (Lamparter *et al.* 1996), the gravitropic response is actively turned off in light by way of the phytochrome. Chlorophyll synthesis is also under phytochrome control. These mutants therefore behave in darkness as though they were growing in light, and are therefore operationally similar to *constitutively photomorphogenic/de-etiolated (cop/det)* mutants of higher plants (Schwechheimer & Deng 2000). The hybrids between these strains (*gtr-308 + gtr-310* and *gtr-310 + gtr-311*) do not show complementation, indicating that *gtr-308*, *-310* and *-311* are all impaired in the same function. Hybrids with all other *gtr* mutants show an aligned upward growth, indicating that this function is different from the functions affected in other *gtr* mutants.

*gtr-310, -303, -304, -410, -414, -428, -445, -446, -502, -505 and -507 (Aligned but growing downwards)*

These mutant strains align to gravity but orient downwards instead of upwards. They form a homogeneous group, hybrids between group members all having the same phenotype (aligned but growing downwards). The group members are therefore likely to be loss-of-function mutants in the same gene.

*gtr-403, -406, -408, -415, -421 and -444 (Poorly aligned)*

These mutant strains all show little or no alignment to gravity. Where hybrids have been obtained between these strains, some have phenotypes similar to the component strains (*gtr-403 + gtr-415*, *gtr-406 + gtr-415*, *gtr-408 + gtr-415*), while two hybrids (*gtr-406 + gtr-408* and *gtr-408 + gtr-421*) have an aligned, downward growing phenotype. Thus, no hybrid between mutants having a near-agravitropic phenotype shows complementation to give a wild-type phenotype. The significance of the phenotypes of these hybrids is discussed more fully as follows.

*Hybrids between downward growers and agravitropic mutants*

The phenotype of hybrids between near-agravitropic mutants and mutants that align to gravity correctly but orient downwards is consistently similar to the downward-growing component (see *gtr-303 + gtr-403*, *gtr-303 + gtr-406*, *gtr-303 + gtr-408*, *gtr-303 + gtr-415*, *gtr-303 + gtr-421*, *gtr-304 + gtr-408*, *gtr-406 + gtr-445*, *gtr-408 + gtr-505*, *gtr-421 + gtr-428*, *gtr-428 + gtr-444*). Had strains with these two contrasting phenotypes resulted from loss of different functions, it would be expected that hybrids between them would have a wild-type phenotype (aligned growth in an upward direction). The finding that agravitropic mutants cannot complement mutants having the downward growth pheno-

type strongly suggests that these mutants are deficient in overlapping functions, with an aligned downward growth representing partial loss of function (i.e. loss of the ability to orient their gravitational response correctly), while agravitropism represents the loss of both orientation and alignment. This pattern is consistent with two explanations:

- 1 It could be that independent genes are responsible for the alignment and orientation and that mutants that show symmetrical growth are mutants in both genes. The inability to align would be epistatic to the ability to orient.
- 2 Alternatively, a single gene product could be responsible for both orientation and alignment. Mutants having a near-agravitropic phenotype would have lost both functions, while the well-aligned downward growers would have lost only the ability to orient.

The first explanation would require all alignment-impaired mutants to be double mutants. This is only likely if the two genes concerned are closely linked and if for some reasons, deletions involving both genes are common. This scenario is possible, but we think it unlikely. Furthermore, a further observation leads us to favour the second explanation. The phenotype of two of the hybrids between alignment-impaired mutants (*gtr-406 + gtr-408* and *gtr-408 + gtr-421*) is an aligned, downward-growing phenotype, and thus the hybrids show partial complementation. This pattern of complementation, where two mutants belonging to the same complementation group complement to give a less than wild-type phenotype, was originally termed intracistronic complementation, but is now better termed interallelic complementation. It has been shown in microbial systems that it can result from the mature gene product being a multimer containing two or more subunits coded for by the same gene (Fincham & Coddington 1963; Garen & Garen 1963; Schlesinger & Levinthal 1963). The product of complementation between two mutants would be a heteromer, which may have a partial activity. Because two mutants that have lost their ability to align complement to give an aligned but downward growing phenotype, it is likely that the later is a partial activity. The data therefore favour the interpretation that a single gene controls both the direction and the fidelity of the gravitropic response, and that it may contribute more than one copy of a subunit of the functional product.

*gtr-506 (Poorly aligned)*

Hybrids between this mutant and wild-type strains have a wild-type phenotype, and so this mutant's gravitropic phenotype (poorly aligned, upward growth) is recessive. It also complements *gtr-503*, and so is functionally distinct from this mutant. Its complementation pattern with mutants of the large group comprising both aligned downward growers and near-agravitropic mutants is complex. In no case is the hybrid phenotype wild-type, but some hybrids between *gtr-506* and agravitropic mutants show a near-wild-type phenotype (moderately aligned upward growth, e.g. *gtr-406 + gtr-*

506), while others show a near-agravitropic phenotype (e.g. *gtr-421 + gtr-506*). Hybrids between *gtr-506* and aligned downward-growing mutants also have a range of phenotypes; the *gtr-410 + gtr-506* hybrid is more poorly aligned than either component, while the *gtr-502 + gtr-506* hybrid has a near-wild-type phenotype. It is therefore likely that *gtr-506* is another member of the large complementation group and that the range of phenotypes shown by its hybrids with other members is a further result of interallelic complementation.

### Future work

With the exception of *gtr-301* (previously designated as *wwr-1*), no detailed investigation of the physiological and cytological properties of the mutants has yet been undertaken, although it is planned that these should be carried out as soon as resources permit. *gtr-301* has been investigated in detail (Wagner *et al.* 1997) and the zonation of the apical cell does not differ markedly from the wild-type strain from which it was derived (WT3). Plastid sedimentation upon reorientation also occurs in a similar manner to the wild-type. The detailed kinetics of the response to reorientation to the gravity vector differs from the wild-type in the direction of the responses but not in their timing. Thus, during cell division, the wild-type response changes from upward to downward growth, while *gtr-301* changes from downward to upward growth. Wagner *et al.* (1997) concluded that *gtr-301* was not impaired in polarity establishment or the coordination of the gravitropic response, but in the interpretation of the gravity input. There are no obvious differences in the morphology of the apical cells of the other strains described here, but studies employing time-lapse video recording of the response to reorientation need to be undertaken to characterize phenotypes more completely.

In addition to the current work aimed at Mendelian analysis of some of the mutants described here, work is in hand to isolate the genes involved. A gene-tagging programme has been initiated and several potential mutants affected in their response to gravity have been isolated. With the establishment of a detailed genetic map of *C. purpureus* (McDaniel, Willis & Shaw, unpublished results), marker-based cloning may present an alternative method for identifying the genes involved. Parallel studies on the genome of *P. patens* are in progress, with completion of genome sequencing and sequence annotation scheduled for the end of 2006. Although sequencing the genome of *C. purpureus* is not yet scheduled, the molecular similarity of the two species has already been established (see e.g. Sperling *et al.* 2000; McDaniel & Shaw 2005), and the *P. patens* genome sequence is therefore likely to aid studies of *C. purpureus* directly.

### CONCLUSIONS

1 The gravitropic mutants of *C. purpureus* comprise at least three complementation groups.

- 2 Light signals, detected by phytochrome, induce chlorophyll synthesis and inhibit response to gravity. One group, comprising three mutants, continues to make chlorophyll but is insensitive to gravity in darkness; phenotypes consistent with members of this group behaving in darkness, as though they were in light.
- 3 The remaining mutants comprise two complementation groups, one with a single member, the other with at least 17 members.
- 4 The larger group contains members some of which are insensitive to gravity and others which align to gravity, but orient their growth downwards.
- 5 The complementation patterns of this larger group are consistent with them, all being mutant in a single gene the product of which is needed both to orient and align the gravitropic response.
- 6 We hypothesize that the product of this gene is likely to contribute more than one subunit to a mature functioning complex.

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