GRAVITROPIC REACTIONS OF THE MUSHROOM FRUIT BODY OF *COPRINUS CINEREUS*

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Abstract

Gravitropic bending in Coprinus cinereus stipes (= mushroom stems) is a two-stage process, the first stage being an elastic deformation, the second a rigidification process. This seems to be different from growth processes concerned with normal extension of the stipe. Control of gravitropic bending is exercised by the apical half of the *Coprinus* stipe and the same tissues are responsible for gravity perception and gravitropic response. The reaction time is about 21 minutes. Perception of the gravity vector does not involve modulation of Ca^{2+} metabolism, proton channels nor stretch-activated ion channels. The perception time is 7 minutes. Gravistimulation is not additive and the angle of bending attained depends on gravistimulation dose (implying that sustained stimulation is required for sustained bending). There is no relationship between extent of gravistimulation and rate of response or reaction time.

1. Introduction

Most of our research efforts are devoted to the study of fungal developmental biology but the majority of the morphogenetic events we deal with are poor candidates for experiment because their control processes are entirely endogenous and difficult to access. We have recently begun to experiment with gravitropism in *Coprinus cinereus* as a morphogenetic model system. The mushroom is a spore dispersal device which is completely dependent on gravity for efficient functioning. Spore release in these organisms is intolerant of water so the mushroom cap is an umbrella from which spores must fall vertically to escape from the protecting cap, eventually to be dispersed on air currents. Spores often fall for many millimetres and usually with little clearance, between gills or through the tubes of the fruit body, so it must be positioned absolutely vertically if spores are to escape successfully. This positioning can only be achieved by referencing morphogenesis to the gravity vector.

Though interesting in its own right, the gravitropic response is a simple developmental pattern-forming process. Its control demands that the organism has a gravity perception system and a means of coupling this to control differential tissue growth. Study of gravitropism is therefore a natural, non-invasive means of generating a particular morphogenetic change on demand in a specific location. We are using gravitational biology as a tool to study cell and developmental biology.

2. Basic kinetics

Gravitropism is the bending response which is usually assumed to be due to asymmetrical redistribution of growth potential in response to change in the perceived direction of the gravity vector (Moore, 1991). We have used video recording and computer-based video-image analysis to complete the first kinetic analysis of gravitropism of the stipes (= stems) of (mushroom) fruit bodies of the basidiomycete fungus *Coprinus cinereus* (Kher et al., 1992). Completion of meiosis in the cap coincides with the stipe becoming competent to react gravitropically. The gravitropic response in this species is rapid, being evident within 30 minutes of disorientation. The bend is initiated near the apex of the stipe, then becomes more acute and progresses basally, traversing 40% of the initial length of the stipe. Gravitropic bending is most likely the result of asymmetric distribution of growth, as represented by cell expansion, being stimulated by a diffusing, extracellular growth factor produced by the apical region of the stipe. Bending raises the apex and as this approaches an angle of about $35°$ to the horizontal, curvature compensation begins to adjust the degree of bending so that the apex can be brought exactly vertical. Approximately 90% of bending is compensated (i.e. effectively reversed) to bring the stipe to the vertical. This curvature compensation requires that the apical region is free to move towards the vertical (when the apex is restrained to the horizontal, the stipe can bend into a complete circle). Bending and curvature compensation appear to be quite separate processes and the mechanism
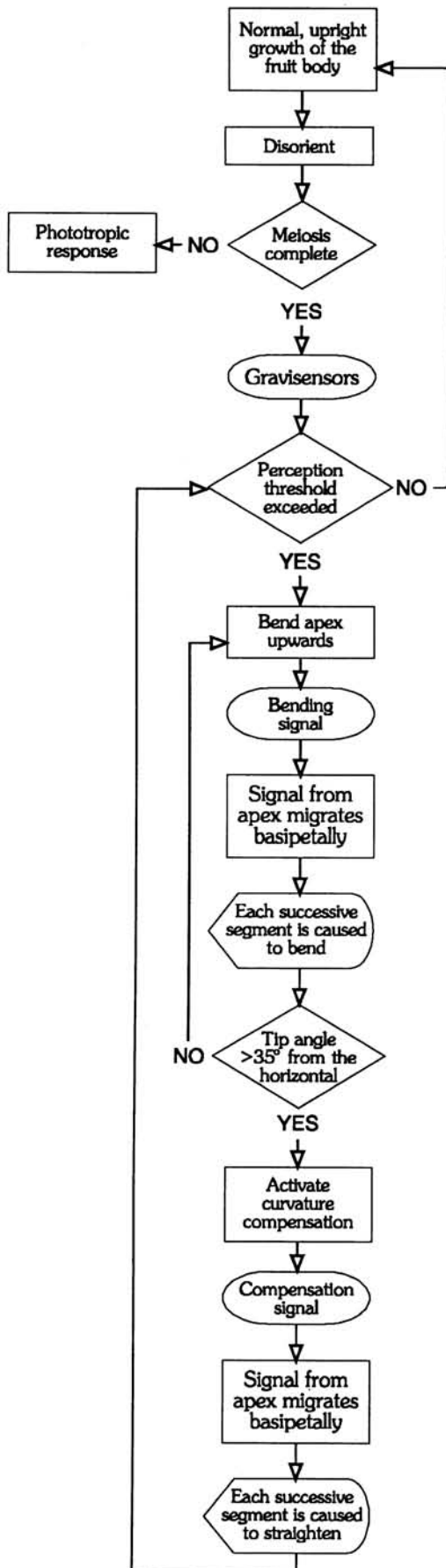
Fig. 1. Flow-chart of the gravitropic response in stipes of *Coprinus cinereus* (from Moore et al., 1994).

involved in curvature compensation is uncertain, but it may entail a second diffusing signal.

This basic description of the phenomenon can be formalised into a flow-chart (Fig. 1). The resultant model (Moore *et al.*, 1994) is adequate at a descriptive level but a number of recent observations suggest that it is not an adequate description of the mechanisms which drive gravitropism. In the rest of this paper we will concentrate on those observations which are most difficult to explain.

3. What constitutes 'the apex'?

As described above, the stipe apex must be free to move for it to regain the vertical and there is an implication in this that the apex may be the sensory focus. However, large segments of the apical part of the stipe can be removed without affecting either the ability of the stipe to show gravitropic bending or its ability to compensate the curvature so induced and adjust to the vertical. In these experiments, apical segments 10, 20 & 30 mm long were removed from 50-60 mm stipes prior to their being laid horizontal in the standard gravitropism assay. Even when more than half of the stipe was removed the remaining segment usually responded gravitropically and usually adjusted to the vertical; i.e. curvature compensation, as well as gravitropic bending, occurred normally in these specimens (Greening, Holden & Moore, 1993). The only consistent influence of apex removal was on the timing of the first visible gravitropic response (= reaction time): the greater the portion of stipe removed, the longer it took for a response to gravity to be observed. Clearly, although the stipe is polarised, the structural stipe apex is not necessary for the gravitropic response to occur, but the amount of stipe excised correlated directly to the reaction time of the stipe.

One way of accounting for this might be to suppose that although the whole of the upper half may contain hyphal cells able to produce the gravitropic 'reaction', the proportion of such gravitropically competent cells is successively reduced in zones further from the true apex. Most of the rest of the kinetics could then be understood in terms of diffusion of a chemical bending signal through the cell population.

4. How is curvature compensation controlled?

Observations show that when the apex of the stipe attains an angle of about 35° to the horizontal, the rate of bending begins to decline. This is the first expression of the curvature compensation which eventually adjusts the apex to the vertical (Fig. 1). A facile explanation of this is that the apex in some way senses its orientation and controls the bending contour by producing growth factors which either diffuse or are translocated to their site of action a few
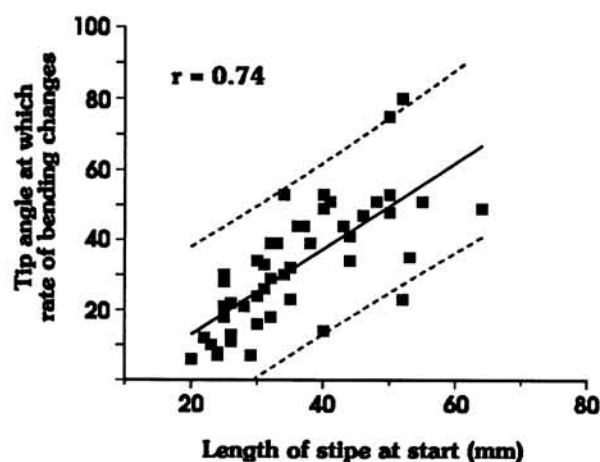
Fig. 2. Relation between initial stipe length and angle of onset of curvature compensation.

Fig. 3. Relation between initial stipe length and time of onset of curvature compensation.

cm below the apex. Unfortunately, this simple model would not predict a relationship between the initial length of the stipe and the angle of the apex at which the rate of bending changes; but these two features are correlated (Fig. 2).

It is not at all clear why the initial length of the stipe should determine the angle at which the bending rate declines. The longer stipes could obviously exert a greater turning force about the point of bend which might be important if a mechanical feedback regulates bending. However, response to change in the distribution of mass has been excluded as a controlling factor by demonstration that application of lateral loads of up to 20 g had no adverse effects on adjustment of stipes to the vertical, nor on their continued vertical growth (Greening et al., 1993). Further, one would predict a negative correlation for such a mechanism (longer stipes would be expected to reduce bending rate at a lesser angle than short stipes); but the correlation has a positive slope.

Stipe length increases as the fruit body matures, so it is a measure of developmental age. Since it is known that the rate of stipe extension growth increases as the fruit body matures (Hammad et al., 1993; and see Table 1) it might be argued that the observed correlation is due to the faster extension of the longer stipes. However, faster extension would surely mean that the angle at which the rate of bending changes would be attained sooner; yet there is no correlation between the time at which the rate changes and the initial length of the stipe (Fig. 3). We are unable to explain this peculiarity, but suggest that it implies that the apex is not responsible for sensing its orientation. Rather, this essential evaluation is performed by a considerable region of the upper part of the stipe.
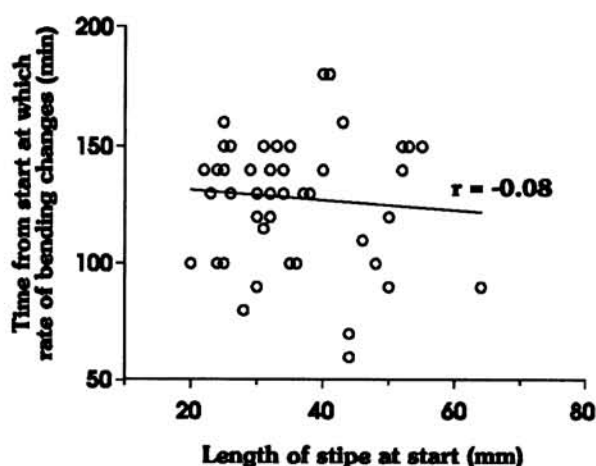
5. Is gravitropic growth different from normal extension growth?

We can demonstrate some dependence on developmental age, however. Recognising that this occurs is an important aspect of experimental design (fruit bodies of similar *developmental* age must be chosen for experiment to minimise dispersion in the numerical data obtained), but it also raises the possibility that the growth differential which generates the gravitropic bend is somehow different from the growth differential responsible for extension growth of the stipe. The data are shown in Table 1. Note that parameters related to gravitropic bending (reaction time (= time to first appearance of the bend), rate of bend movement towards the stipe base, final angle of the apex) all show a steady change throughout the six-hour harvesting period, whilst the extension growth of the stipe shows a distinct maximum in the middle of the harvesting period, implying that the growth processes responsible for the two phenomena are different.

Other indications that tropic bending may not result from a simple redistribution of the normal growth potential of the stipe come from work with the calcium ionophore A23187 and morphometric analyses of stipe cell size distributions (see below).

6. Dosage-response relationships

The sensitivity to gravitational stimulation has been determined using 2 r.p.m. clinostat rotation to remove partially-stimulated stipes from the normal unidirectional gravitational field (Hatton & Moore, 1992). The presentation time, i.e. the minimum time of stimulation required to elicit a gravitropic reaction, has been established to be 7 minutes, this

Table 1. Growth and gravitropism of stipes of *Coprinus cinereus* harvested at different times of day

	Time of harvest		
	11.30	15.00	18.20
Extension growth rate (μm min^{-1})	36.1	64.6	36.8
Increase in stipe length in 3 h (%)	17.7	32.6	18.8
Reaction time (min)	89.8	37.0	31.1
Rate of bend movement towards the stipe base (μm min^{-1})	45.8	59.8	72.2
Tip angle attained after 3 h (°)	23.1	37.0	62.7

Entries show mean values from 8 replicates.

being the first determination of a presentation time for any fungal gravitropic response. The extent of the gravitropic response, measured as the angle of the stipe apex at maximum curvature, was dependent upon the gravitational exposure time (Hatton, 1993). The reaction time did not depend on exposure time and exposures were not additive. Immediately after reaching maximum curvature, stipes placed on the clinostat after various gravity exposure times 'relaxed' by 5°.

These observations suggest (i) that the gravitropic impulse is an 'all-or-nothing' signal in *Coprinus cinereus*, (ii) that sustained exposure to the unidirectional gravity vector is necessary for the normal gravitropic response, (iii) that perception and response probably occur in the same tissue regions, and (iv) that gravitropic bending is a two-stage process with an initial, reversible, phase of elastic bending.

7. The problem of perception

Control of cell calcium accumulation has been implicated in the gravity perception mechanism of plants but no attempt has ever been made to assess its role in fungal gravitropism. Using concentrations and treatments known to eliminate gravitropism and other tropisms in plant organs, the role of Ca^{2+} in the gravitropic mechanism was examined by exposing stipes to a Ca^{2+} channel blocker, verapamil; a Ca^{2+} ionophore, A23187; a Ca^{2+} chelator, BAPTA; or calmidazolium, an inhibitor of calmodulin-mediated Ca^{2+} uptake (Novak Frazer & Moore, 1993). These inhibitors had no effect on gravity perception but the ionophore (which enhanced stipe extension), the chelator and calmidazolium (which had no effect on stipe extension) all significantly diminished the gravitropic response. It is noteworthy that the ionophore, A23187, enhanced stipe extension growth rate (by 30%) but decreased the rate of response, i.e. rate of bending, by 43%. This suggests that tropic bending may not result from a simple redistribution of the normal growth potential of the

stipe (see above). Overall, the results of these treatments would be consistent with regulation of the gravitropic bending process requiring accumulation of Ca^{2+} within a membrane-bound compartment, but it seems that Ca^{2+} is not involved in gravity perception.

Indeed, no treatment applied so far has inhibited perception. Inhibitors of stretch-activated (mechano-sensitive) ion channels, gadolinium chloride and amiloride hydrochloride, have been applied and found to impair the gravitropic response only at concentrations which inhibited stipe extension. Experiments with 2,4-dinitrophenol (a protonophore and alkali metal channel) had a similar outcome.

At the moment, therefore, the basis of the perception mechanism is obscure, but we are making progress in understanding the signal transduction pathway. Early experiments suggested that a growth factor might diffuse or be transported from the gravisensitive apex towards the base to cause upwards curvature (Kher *et al.*, 1992). Although no such growth factors or hormones have so far been isolated from any higher fungi, we have developed a method to extract compounds which cause vertical stipes to bend. There appear to be two components which have recognisably different activities in the bioassay. These we call Fungiflex-1 and Fungiflex-2. Though the preparations are still impure, FT-IR and NMR analyses are giving useful results and a structure determination seems accessible in the near future (Novak Frazer, unpublished observations).

8. What makes stipes bend?

Work is in progress on a morphometric analysis of the cell patterning which achieves the gravitropic curvature. *Coprinus* stipes contain two populations of hyphae: narrow and inflated (Hammad, Watling & Moore, 1993). During normal vertical growth of *Coprinus* stipes, there is evidence that inflated hyphae inflate further and that the proportion of narrow hyphae declines as the stipe grows from 45

Table 2. Morphometric analysis: cross sectional areas of individual narrow and inflated hyphae during normal vertical growth and during gravitropic bending of stipes of *Coprinus cinereus*

	Mean cross sectional area (μm^2)		
	Narrow hyphae	Inflated hyphae	% narrow hyphae
Gravitropically bent stipes			
Upper region of bend	9.4	183.8	34.8
Lower region of bend	9.4	182.2	35.6
Normal vertical growth			
45 mm stipe apical region	9.2	202.3	36.2
70 mm stipe apical region	9.6	246.0	26.3
45 mm stipe middle region	8.2	181.2	37.8
70 mm stipe middle region	9.9	221.1	24.2
45 mm stipe basal region	8.8	286.7	32.8
70 mm stipe basal region	8.4	226.8	38.3

Entries are means of measurements of between 116 and 535 cells in randomly-chosen transects across radii of transverse (light microscope) sections. Methods are detailed in Hammad, Watling & Moore (1993).

Table 3. Comparison of the gravitropic phenotypes of fruit bodies formed by the normal dikaryon and those formed by two $A_m B_m$ mutant homokaryons

	Dikaryon	$A_m B_m$ strain 1	$A_m B_m$ strain 2
Mycelial growth rate ($\mu m\ h^{-1}$)	206	148	160
Stipe extension (% in 3 h)	18.2	18.8	17.5
Reaction time (min)	27.7	43.8	38.2
Tip angle attained after 3 h (°)	57.3	55.6	54.3

to 70 mm (Table 2), indicating that stipe extension involves both an increase in cross-sectional area of inflated hyphae and recruitment of narrow hyphae into the inflated population. Similar analyses of the upper and lower regions of the bent zones in gravitropically-responding stipes show convincingly that neither cell cross-sectional area nor cell-size population structure changes during bending (Table 2). This analysis is not yet complete. In particular, similar measurements are required of cell length in longitudinal sections, but even the observations in Table 2 are sufficient to suggest strongly that the growth mechanism which causes gravistimulated stipes to bend is different from that which generates extension growth. The observations also provide a contrast with plant stems where increases have been recorded in both length and diameter during gravitropic bending (Sliwinski & Salisbury, 1984).

9. Conclusions

At the beginning of this research we took as our starting point the possibility that gravitropic bending of mushroom stipes might resemble gravitropic bending of plant roots and stems. So little was known about the fungal system that it seemed sensible to expect that analogies might exist between linear organs comprised of linearly-arranged cells with rigid walls. A major out-turn of the experiments, therefore, is the catalogue of differences between fungal and plant strategies of gravitropism.

Perception of the gravity vector stimulus does not involve modulation of Ca^{2+} metabolism in *Coprinus*, nor does it involve proton channels or stretch-activated ion channels.

The mushroom stipe is polarised, with the apical zone being responsible for modulating gravitropic bending. However, the 'apical zone' extends over more than half the length of the *Coprinus* stipe. There is also reason to believe that the same tissues are

responsible for gravity perception and gravitropic response.

Sub-minimal gravistimulatory exposures are not additive in *Coprinus* and there is no relationship between extent of gravistimulation and either rate of response or reaction time. Instead, the maximum angle of bending attained depends on gravistimulation dose (implying that sustained stimulation is required for sustained bending).

Bending appears to be a two-stage process in which an initial (spontaneously reversible) elastic deformation is followed by a rigidification process, and the asymetric growth differential responsible for gravitropic bending appears to be different from, and independent, of the normal extension growth of the stipe.

Many of these points are based solely on interpretations from indirect experiments. They can only be proved by a genetic dissection of the gravitropic reaction in *Coprinus*. All of the experiments reported so far have made use of a strain isolated from nature. This is a normal dikaryotic strain (i.e. two parental homokaryons came together and mated at some stage in the past to form the fertile dikaryon in which each cell contains one haploid nucleus from each parent). Genetic analysis (conventional and molecular) of such strains is difficult because the cells are analogous to a diploid. Fortunately, we have cultures of $A_m B_m$ mutants which are homokaryons able to produce fruit bodies as the result of mutations in the incompatibility factors. These are ideal candidates for the induction of developmental mutations because any recessive variants induced are immediately expressed. Phenotypic comparisons of fruit bodies formed by the dikaryon and two such $A_m B_m$ mutants reveal only minor differences (Table 3) and a programme aimed at isolating gravitropic mutants is to be started in the near future.

10. References

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