The Gravireceptor of *Phycomyces*

Its Development Following Gravity Exposure

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ABSTRACT The gravitropism of a mature stage IV *Phycomyces* sporangiophore has a shorter and more uniform latency if the sporangiophore is exposed horizontally to gravity during its earlier development (stage II and stage III). This early exposure to an altered gravitational orientation causes the sporangiophore to develop a gravireceptor as it matures to stage IV and resumes elongation. A technique has been developed to observe the spatial relationship between the vacuole and the protoplasm of a living sporangiophore and to show the reorganization caused by this exposure to altered gravity. Possible gravireceptor mechanisms are discussed.

INTRODUCTION

The gravitropism of *Phycomyces* sporangiophores has become an indispensable part of contemporary investigations of the photoresponses of this organism because gravitropism can be so conveniently balanced against phototropism. For example, the photogeotropic equilibrium action spectra (Galland, 1983), using wild-type and mutant strains, have provided important new information suggesting a complex photoreceptor whose components can be genetically distinguished. Genetic studies of phototropism (Lipson and Terasaka, 1981) and the testing of quantitative phototropism models (Medina and Cerdá-Olmedo, 1977) have similarly depended strongly on the existence of gravitropic sensitivity in the sporangiophore.

In spite of the practical importance of gravitropism, its cellular mechanism is almost completely unknown. The persistence of gravitropic bending in sporangiophores submerged in a dense liquid established that the primary gravireceptor is not connected to the stresses in the external cell wall set up by the weight of the massive sporangium (Dennison, 1961). Since the sporangiophore does not contain dense statoliths such as are usually associated with gravitropism in the roots and shoots of higher plants, there is no obvious intracellular structure that could function analogously in *Phycomyces*. An additional problem is caused by the weak and variable nature of the gravitropic response of *Phycomyces*, noted by

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investigators for more than a hundred years (see, for example, Sachs, 1879). The use of a centrifuge to increase the stimulus strength greatly increases the speed and uniformity of the response (Dennison, 1961), but the great variability in the geotropic response at 1 *g* remains a disquieting reminder that other factors affecting gravireceptor function may remain to be discovered.

Our basic view of the gravireceptor mechanism is that it must involve the rearrangement of intracellular liquid phases of differing density, specifically the protoplasm and vacuole. Although the densities of these phases have not been measured in *Phycomyces*, comparable data from *Nitella* (Kamiya and Kuroda, 1957) indicate that the cytoplasm is 0.3–0.5% denser than the vacuole. Given such a density difference, gravity acting on a horizontal cell would bring about an upward buoyant force on the vacuole with respect to the surrounding layer of protoplasm. The gravity stimulus could arise from the existence of this buoyant force or from the resulting displacement of these phases relative to each other.

The gravireceptor signal might arise solely from the asymmetry of protoplasmic thickness: since the lower layer is thicker, it might cause extra cell wall synthesis merely by the corresponding excess production of cell wall precursors. A correlation between curvature and protoplasmic thickness was noted in *Phycomyces* by Kohl (1885), but he found that the protoplasm was thicker on the upper, concave side of a sporangiophore that had shown upward (negative) gravitropic bending. This finding is at odds with our prediction.

Alternatively, the primary stimulus might result from an asymmetry of intracellular organelles, caused by a shift in the vacuole. An analogous mechanism seems to hold in *Chara* rhizoids, which are positively gravitropic. The tip of the rhizoid contains statoliths, which quickly sediment to the lower wall of a cell placed on its side and in turn cause a displacement of Golgi vesicles to the upper surface. This asymmetry causes a greater incorporation of Golgi vesicles into the plasma membrane of the upper surface, resulting in a greater growth rate of the upper wall and a net downward curvature (Sievers and Schröter, 1971). Such a model is difficult to apply to *Phycomyces*, with the vacuole playing the role of statolith, because there is no evidence up to now that a vacuole shift actually occurs in a horizontal cell (Banbury, 1962). Until such an asymmetry can clearly be demonstrated, it is premature to postulate a gravireceptor mechanism along these lines.

Why has it been so difficult to observe intracellular rearrangements caused by gravity? Perhaps such changes occur most readily at certain developmental stages during sporangiophore maturation. In the most immature state (stage I; Errera, 1884), the sporangiophore is without a sporangium, and in its upper portion there is a solid plug of protoplasm (Kirchheimer, 1933), whereas below this there is a vacuole surrounded by a cylindrical protoplasmic sheath. In Errera's stage II, growth stops and sporangium formation begins, with much protoplasmic streaming into the swelling tip (Keene, 1919; Swingle, 1903). In stage III, neither the sporangiophore nor the sporangium grows, but spore differentiation begins. In stage IV, sporangiophore growth resumes, and the sporangium darkens from yellow to brown to black; internally, the spores and the columella wall mature (Errera, 1884; Keene, 1919; Swingle, 1903). The key point is that there is a solid protoplasmic plug in stages I, II, and III, but eventually in stage IV it must

disappear, since in mature stage IV sporangiophores the vacuole is usually continuous from the columella to the basal plug. Might not the cell have a quite different, perhaps greater, sensitivity to gravity in one of these immature stages? In particular, could the plug of protoplasm be shifted much more readily by gravity than the cyclindrical protoplasmic sheath of a mature cell? If this is so, then one might expect that a sporangiophore would be far more sensitive to a gravity stimulus if it were exposed while still immature, i.e., in stage II or III or early stage IV.

This is precisely what is suggested by the experiments of Pilet (1956), who placed sporangiophores on their sides at various stages of maturity (as shown by height) and measured their growth and the development of gravitropic curvature by means of shadowgraphs. His data show clearly that the reaction time (time for 50% of the specimens to bend 5° or more) increases from 15 to 60 min as the initial sporangiophore height increases from 4 to 9 mm. Under Pilet's culture conditions, the height of stage III sporangiophores varied from 3.5 to 6 mm, a representative value being ~4.5 mm, and therefore his sporangiophores of height 4 mm are mostly in stage III or early stage IV. Sporangiophores higher than this are almost all in later stages of maturity. Pilet's results thus show that sporangiophores in stage III and early stage IV react more quickly to gravity than do those in later stages. In other words, during an early developmental stage, the sporangiophore is uniquely sensitive to gravity and, if exposed horizontally during this stage, might be said to acquire a gravireceptor that confers increased sensitivity to gravity in subsequent stages.

The purpose of our paper is twofold. First, we will present evidence that the latency of gravitropism is shorter and more uniform if the sporangiophore has been exposed to lateral gravity in an early stage. Second, we will show that while the sporangiophore is immature, the intracellular organization is highly sensitive to transverse gravity exposure, which produces a considerable alteration in the position of the vacuole. Such a vacuole shift, with its accompanying alterations in protoplasmic sheath thickness, may be related to the primary mechanism of gravireception.

METHODS

Cultures

The wild-type (−) strain of *Phycomyces blakesleeanus* (no. 1555 of the U. S. Northern Regional Research Center, Peoria, IL) was obtained from the California Institute of Technology, Pasadena, CA. Vegetative spores were heat-shocked at 45°C for 10 min and inoculated at a density of five spores per culture vial. Each vial, 12 mm in diameter, was filled with a sterile medium containing 4% potato dextrose agar (0013; Difco Laboratories, Inc., Detroit, MI), 0.1% Wesson oil, and 0.05% thiamine hydrochloride. Immediately after inoculation, the vials (in loosely covered jars) were placed in painted wooden culture boxes illuminated from above, and after 3 d the covers were removed. Mature stage IV sporangiophores were routinely selected from 4- or 5-d-old cultures (on occasion from cultures ranging up to 11 d old) and were 25–30 mm in total height.

We used either fluorescent illumination (GE 40-W cool-white, fluence rate 0.27 $W \cdot m^{-2}$ in the spectral range 300–800 nm) or incandescent illumination (GE 7.5-W refrigerator lamp, fluence rate 0.23 $W \cdot m^{-2}$ in the spectral range 300–800 nm) in the culture

boxes, since we were investigating the possible effects of culture illumination intensity and quality on the physiological properties of sporangiophores. Illumination is known to affect sporangiophore initiation (Bergman, 1972; Thornton, 1975), and it has been reported (Dennison and Foster, 1977) that sporangiophore growth rates increase when the culture box illumination is changed from three 15-W incandescent lamps to two 7.5-W lamps. Although there are a number of differences between incandescent-grown and fluorescent-grown sporangiophores (e.g., the latter are 20% shorter at stage II and have about twice the content of beta-carotene), no difference in their gravitropic response has been found. Consequently, we report here the combined results from sporangiophores grown under both types of illumination.

To produce gravity pre-stressed sporangiophores, we used a gravity stress box, a culture box which is tilted so that the illumination strikes the cultures from 20° below horizontal, thus forcing the sporangiophores to pass through all their developmental stages in a horizontal orientation. For experiments beginning from photogeotropic equilibrium, stage II sporangiophores were placed horizontally in the tropostat overnight, with a broad-band blue illumination source (15-W incandescent lamp plus Corning 5-61 filter [Corning Glass Works, Corning, NY], fluence rate 1.4×10^{-4} W·m^{-2}) striking the sporangiophore from 20° below horizontal.

Tropostat

A tilting stage, based on the tropostat design of Dennison (1965), was used to maintain the sporangiophore growing zone in a fixed orientation relative to gravity. A single culture vial was mounted in a Plexiglas box (23 cm × 23 cm × 13 cm) on an arm that pivoted along a horizontal axis and was connected to an angle scale. A horizontal microscope, fitted with cross hairs and a goniometer eyepiece, was aimed along this same axis, and red illumination (1259 red filter; Corning Glass Works) was focused on a white card behind the specimen. In the operation of the tropostat, the sporangiophore growing zone was lined up on the microscope cross hairs (which can be set horizontal or at some other angle) by tilting the arm holding the sporangiophore. The arm angle was recorded, and the process was repeated after a certain interval. The angle through which the sporangiophore was bent during this interval was simply equal to the change in the arm angle. Since each successive setting returned the sporangiophore growing zone to the original orientation, the gravity stimulus was thereby kept constant throughout the run. In some experiments, the sporangiophore was not reset after each interval and the angle was measured from the horizontal using the goniometer microscope.

Bilateral Rotator

Fig. 1 shows an optimal arrangement designed to deliver phototropically neutral blue illumination to a stationary sporangiophore, which can be fixed in any desired orientation with respect to gravity. In this device, the sporangiophore was illuminated by a pair of opposite beams of equal intensity that can be rotated continuously at 2 rpm, resulting in the average equivalent of a 360° equatorial ring of light. This optimal arrangement consisted of an incandescent lamp whose output was filtered through a Corning 5-61 blue filter and two Schott KG-1 heat-blocking filters (Schott Optical Glass Inc., Duryea, PA) and then focused on an opal glass diffuser. Two mirrors were positioned where they received light from the diffuser and reflected it toward the sporangiophore in two opposing beams. The rotation of these beams was accomplished by a synchronous motor that revolved the diffuser-mirror assembly at 2 rpm. The entire apparatus, including a three-way stage (not shown in Fig. 1) that held the sporangiophore culture vial, was mounted on a rigid beam (not shown in Fig. 1) that pivoted around a horizontal axis. In

a typical experiment, a sporangiophore was placed in a vertical orientation and adapted to the rotating bilateral illumination for 2 h. The gravity stimulus was begun by tilting the apparatus through 90°, which reoriented the sporangiophore from vertical to horizontal without altering the illumination in any way. The gravitropic bending was then followed in red light (incandescent lamp, red Plexiglas filter [Rohm and Haas Co., Philadelphia, PA], two Schott KG-1 heat-blocking filters), using a horizontal microscope fitted with a goniometer eyepiece. In many of the experiments, four culture vials were

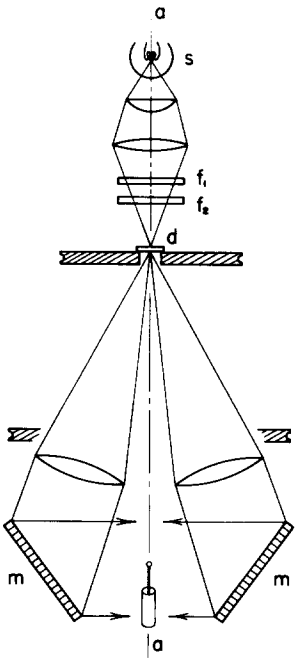

FIGURE 1. Bilateral rotator. Symbols: a–a, axis of 2-rpm rotation of diffuser-mirror assembly; s, source; f_1, heat-blocking filters; f_2, blue glass filters; d, diffuser; m, mirror. A sporangiophore in its culture vial is shown in position, where it is illuminated by the two opposing light beams. The entire apparatus can be oriented with its axis vertical or horizontal. A chamber is formed around the sporangiophore by enclosing the mirror-diffuser assembly in a thin plastic bag which is humidified.

clustered closely together so that several sporangiophores could be monitored simultaneously. Later, it was found that the gravitropic response of the lower specimens was slower than that of the upper ones, probably because of the action of a long-distance avoidance response (Gamow and Böttger, 1982; Lafay and Matricon, 1982). Consequently, only the data from the upper sporangiophores are reported here.

Optical Chamber

Optical distortion of the microscopic image of this cylindrical cell can be minimized by immersion in an inert fluid fluorochemical of matching refractive index, in which growth continues at normal rates for many hours (Shropshire, 1962). We used perfluorotributylamine (fluorochemical FC-43 of the Minnesota Mining and Manufacturing Co., St. Paul, MN). Since it was necessary to observe the cell horizontally from the side, an

Ortholux II photomicroscope (E. Leitz, Inc., Rockleigh, NJ) was mounted with its optical axis horizontal and its stage in the vertical plane. The sporangiophore was mounted in a glass chamber constructed for this purpose from a large microscope slide, to which a glass platform was cemented (Fig. 2). The circular cover glass was supported by an O-ring gasket so that the sporangiophore could rest on the platform and still have a free space between it and the cover glass, while the sporangium hung over the edge of the platform. Since in use this chamber is oriented in the vertical plane, the cover glass must be tightly clamped to the slide with a large brass washer and steel springs. The immersion fluid was pipetted into the assembled chamber through a small gap cut in the O-ring. To load the chamber, a sporangiophore of 12–15 mm in length was selected and cut from the culture medium with a 2-mm cube of medium (and mycelium) left attached at the basal end. The sporangiophore was then placed on the platform with the sporangium projecting free and not far from the center of the viewing field of the chamber, and with the basal end (with cube) also projecting free from the platform but positioned near the edge of the viewing field. Finally, the cover glass was clamped in place, the chamber was tipped vertical, and the fluid was added, leaving a small air space at the top. For photomicrography, we used a 10-magnification objective, an MPS55 camera (Wild Heerbrugg Instruments Inc.,

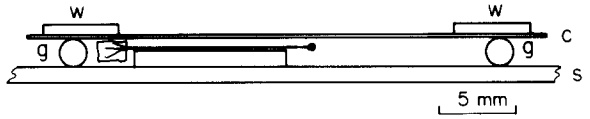

FIGURE 2. Optical chamber seen in cross section. Symbols: s, microscope slide; g, O-ring gasket; c, cover glass; w, washer. A sporangiophore is shown with a small cube of mycelium attached. Not shown are the springs that clamp the washer to the slide.

Farmingdale, NY), and Kodak 35-mm film (Plus-X, developed in Microdol-X according to the manufacturer's instructions). Measurements were made directly from enlargements at a total magnification of 300.

RESULTS

The gravity pre-stress treatment is the placement of a stage II sporangiophore in a horizontal position until its development has progressed to stage IV, which typically requires 4–6 h (Errera, 1884). This can be done by placing the (stage II) sporangiophore horizontally in the tropostat and illuminating it (broad-band blue, 1.4×10^{-4} W·m^{-2}) from 20° below horizontal overnight (~16 h). When the sporangiophore matures and begins to grow, it is in photogeotropic equilibrium with an approximately horizontal orientation; the gravitropic experiment is begun by switching off the illumination. As shown by the example in Fig. 3*a*, the sporangiophore began to bend upward almost immediately, with a latency of ≤10 min. (The sporangiophore growing zone did not actually tilt upward, of course, because in this experiment the tropostat was used to maintain a constant orientation of the growing region.)

Alternatively, the sporangiophore can be gravity pre-stressed by growing the cultures in the gravity stress culture box. Fig. 3*c* shows an example of the gravitropic response of such a sporangiophore, which was placed vertically in the rotator and exposed for 2 h to broad-band blue illumination (3×10^{-4} W·m^{-2}).

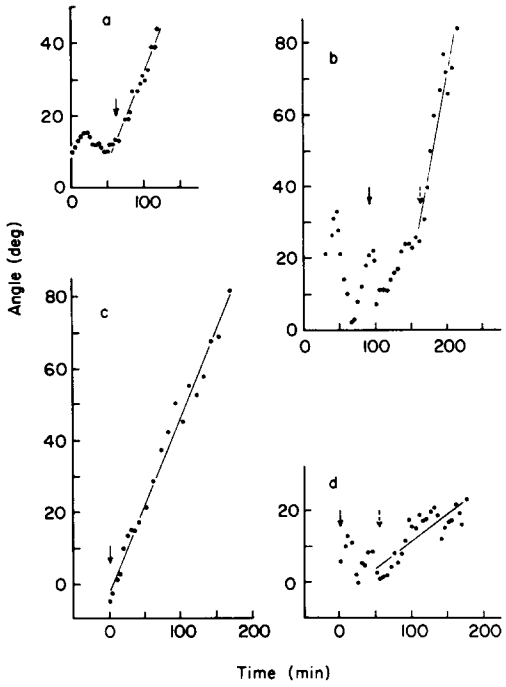

FIGURE 3. Gravitropism, representative experiments. (*a*) Tropostat run, sporangiophore gravity pre-stressed. The sporangiophore was placed horizontally when in stage II and exposed to blue illumination (1.4×10^{-4} W·m^{-2}) at 20° below horizontal. The experiment was begun 16 h later by switching off the illumination (arrow). The angle is measured upward from the horizontal until the beginning of the run; after this, the cumulative tilt angle of the tropostat is added to the initial angle. Fitted line: 0.53 deg·min^{-1}. (*b*) Tropostat run, sporangiophore not gravity pre-stressed. The sporangiophore was placed horizontally when in stage IV and exposed to blue illumination (1.4×10^{-4} W·m^{-2}) at 20° below horizontal. The experiment was begun 3 h later by switching off the illumination (solid arrow). The angle is measured upward from the horizontal until the beginning of the run (solid arrow); after this, the cumulative tilt angle of the tropostat is added to the initial angle. Dashed arrow: estimated beginning of the gravitropic response. Fitted line: 1.12 deg·min^{-1}. (*c*) Rotator run, sporangiophore gravity pre-stressed. The stage IV sporangiophore was selected from cultures grown in the gravity stress box. It was placed vertically in the bilateral rotator and illuminated at 3×10^{-4} W·m^{-2} (broad-band blue) for 2 h. The experiment was begun (solid arrow) by tilting the rotator to the horizontal but keeping the illumination unchanged. The angle is measured upward from the horizontal. Fitted line: 0.50 deg·min^{-1}. (*d*) Rotator run, sporangiophore not gravity pre-stressed. The stage IV sporangiophore was selected from the culture box (not gravity stressed) and placed vertically in the bilateral rotator and illuminated as in *c* above. The experiment was begun (solid arrow) by tilting the rotator to horizontal but keeping the illumination unchanged. The angle is measured upward from the horizontal. Dashed arrow: estimated beginning of the gravitropic response. Fitted line: 0.15 deg·min^{-1}.

At the time shown by the solid arrow, the sporangiophore was tilted horizontally with the illumination kept constant; as before, the gravitropic response began almost immediately. In both of these examples, the gravitropic bending rate was ~0.5 deg·min^{-1}, which is close to the mean value for all the experiments with pre-stressed sporangiophores (see Table I).

In contrast, the omission of the gravity pre-stress treatment caused the appearance of a variable latency before the start of the gravitropic response. For example, Fig. 3*b* shows the response of a sporangiophore that was placed horizontally in the tropostat while in stage IV and left for 3 h during exposure to blue light at 20° below horizontal; the latency in this case was ~70 min. Although the sporangiophore was horizontally exposed to gravity for 3 h, it was no longer in an immature stage, and consequently a longer time was required to show the gravitropic response. Fig. 3*d* shows the corresponding gravitropic

TABLE I

Effect of Gravity Pre-Stress Treatment on the Latency and Rate of Gravitropism

	Mean latency	Mean gravitropic bending rate
	min ± SEM	*deg·min^{-1} ± SEM*
Not gravity pre-stressed (38 experiments)*	61.2±11.5	0.31±0.04
Gravity pre-stressed (17 experiments)‡	13.1±3.9	0.49±0.06

* Stage IV sporangiophores were grown in normal culture boxes. Combined data are from tropostat experiments in darkness and from bilateral rotator experiments at 1.5×10^{-2}, 2.0×10^{-2}, and 3.0×10^{-4} W·m^{-2}.

‡ Data are combined from experiments using sporangiophores from the gravity stress box and using gravity pre-stressed sporangiophores that were placed horizontally in the tropostat while they were in stage II and left in unilateral blue light overnight (~16 h). Two experiments were done using the bilateral rotator at 3.0×10^{-4} W·m^{-2}, and the rest were done using the tropostat in the dark.

response of a stage IV sporangiophore (not gravity pre-stressed) placed vertically in the rotator for 2 h and illuminated as in Fig. 3*c*. Again, the lack of gravity pre-stress caused a latency in gravitropism, in this case ~55 min. In this example, the gravitropic bending rate was reduced; the combined data also show a reduced rate for non–pre-stressed sporangiophores (see Table I). Data from other experiments show that the difference in gravitropic bending rate between Figs. 3*b* and 3*c* is apparently not due to differences in experimental conditions.

The distribution of gravitropic latencies for non–pre-stressed sporangiophores differs strikingly from that for pre-stressed sporangiophores (Fig. 4, *a* and *b*, respectively). Both populations contain a similar distribution of latencies below 30 min but differ strongly above this value; for example, no latencies above 65 min were found for pre-stressed sporangiophores, whereas 34% of the latencies were above 65 min for non–pre-stressed sporangiophores. The latter group shows the classic variability noted in *Phycomyces* gravitropism by Sachs (1879) long ago. Gravity pre-stressing not only produces more uniform latencies, it reduces the mean value as well (Table I).

Our cytological observations provide striking confirmation of the responsiveness of the intracellular organization to gravity, especially when the sporangiophore is immature. As noted earlier, stage I sporangiophores contain an apical protoplasmic plug, which persists through stages II and III, finally to disappear in stage IV. Fig. 5*a* shows the lower end of such a plug, ~1 mm from the sporangium of a stage III sporangiophore placed horizontally ~10 min earlier (this is an approximation of the time consumed in mounting the specimen in the chamber). Note the conspicuous clear mass of material that shifted toward the bottom wall (lower part of the figure); further movement can be seen in Fig. 5,

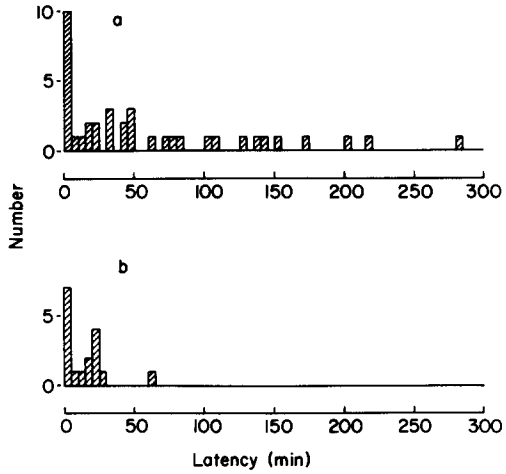

FIGURE 4. (*a*) Distribution of gravitropic latencies for non–pre-stressed sporangiophores. Stage IV sporangiophores were grown in normal culture boxes. Combined data are from tropostat experiments in darkness and from bilateral rotator experiments at 1.5×10^{-2}, 2.0×10^{-2}, and 3.0×10^{-4} W·m^{-2}. (*b*) Distribution of gravitropic latencies for gravity pre-stressed sporangiophores. Data are from combined experiments using sporangiophores from the gravity stress box and using gravity pre-stressed sporangiophores that were placed horizontally in the tropostat while in stage II and left in unilateral blue light overnight (~16 h). Two experiments were done with the bilateral rotator at 3.0×10^{-4} W·m^{-2}, and the rest were done with the tropostat in the dark.

b and *c*. At the same time, the edge of the plug moved further from the cell apex. Finally, as seen in Fig. 5*d*, this clear mass detached itself from the plug and traveled rapidly in the basal direction along the lower wall.

Continued observation of a horizontal sporangiophore has provided evidence that when growth resumes in stage IV, an unmistakable asymmetry develops in the relative thicknesses of the upper and lower layers of the cytoplasmic sheath. For convenience, the thickness of each layer is expressed (Fig. 6) as the total area seen in the optical section photographs, extending from 0.2 to 0.8 mm below the sporangium; these areas are plotted against time in Fig. 6. Note that from 150 to 200 min, the lower layer of the protoplasmic sheath grows thicker, while the upper layer changes little in thickness. Furthermore, the thickening of the

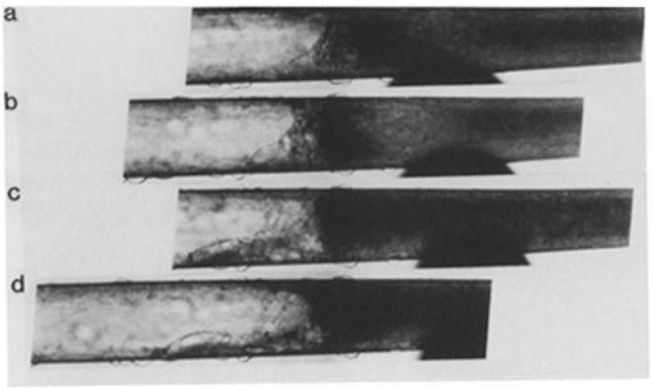

FIGURE 5. Rapid changes in protoplasmic plug. A living sporangiophore in stage II or III (vertically grown) is shown in optical section at various times: (*a*) approximately 10 min after horizontal placement; (*b*) 7 min after *a*; (*c*) 31 min after *a*; (*d*) 34 min after *a*. × 195.

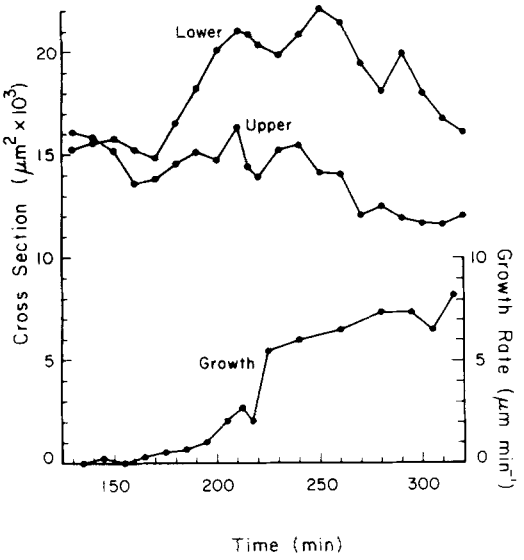

FIGURE 6. Protoplasmic thickness and growth rate. At the top are shown the thicknesses of the lower and upper protoplasmic layers, respectively, of a horizontal stage III sporangiophore that was grown vertically. The values are the total cross-sectional areas, from 0.2 to 0.8 mm below the sporangium, measured directly from the optical section photographs. At the bottom is shown the growth rate, measured from the same photographs. Time is measured from the first photograph, ~10 min after horizontal placement.

lower layer develops in close synchrony with the resumption of the linear growth of the sporangiophore as stage IV begins (Fig. 6, bottom).

DISCUSSION

We have confirmed the work of Pilet (1956) by showing that exposure to gravity in stage II or stage III allows an earlier gravitropic response. In developmental terms, we may say that this horizontal placement alters the differentiation of the cell, causing it to develop a high gravireceptor sensitivity, as reflected by a shorter latency and a more rapid rate of response. The permanence of this change is not yet established, however. Our data do not contradict the concept of irreversible gravireceptor development, but we have not investigated the persistence of gravireceptor sensitivity during long periods of vertical orientation.

Our results make it possible to increase greatly the uniformity of the gravitropic response, either by growing the cultures in a gravity stress box or by placing them in a horizontal position while they are still in stage II. It is hoped that this improved technique will prove useful in further investigations of the mechanism of *Phycomyces* gravitropism.

The gravireceptor can develop without horizontal exposure during the cell's immature stages, however. For example, ~50% of the non–pre-stressed sporangiophores showed little or no gravitropic latency (Fig. 4*a*). This could be due to a chance asymmetry in the protoplasmic sheath, which, under the action of gravity, would cause the thicker (and hence heavier) part of the sheath to slide to the lower wall within a few minutes (required by the short latencies observed in some non–pre-stressed sporangiophores). To support this hypothesis, it would have to be shown (*a*) that there are chance variations in the protoplasmic sheath thickness of appropriate magnitude in vertically grown cells, and (*b*) that such an asymmetric sheath reorients within a few minutes. Photogeotropic equilibrium experiments typically begin with vertically grown stage IV sporangiophores, which are placed in horizontal illumination for as long as 10 h (Galland, 1983). The uniformity of the endpoint in such experiments is truly remarkable and suggests that the gravireceptor does indeed develop reproducibly under these circumstances. Thus, we suggest that the gravireceptor can develop by any one of three pathways: slowly by maturation during horizontal exposure in stage III, quickly by the horizontal exposure of a randomly asymmetric protoplasmic sheath in stage IV, or very slowly by horizontal exposure in stage IV, in photogeotropic equilibrium. Whatever the pathway, the endpoint would be the same, however.

The two distributions in Fig. 4 show an interesting similarity for latencies shorter than 30 min. Each is strongly bimodal, with a prominent sharp peak for 0–4 min latency and a broad maximum for 20–40 min latency. The latter feature is consistent with the 30-min latency observed by Dennison (1961) using centrifugal stimuli, and it may reflect a fundamental gravireceptor time constant. The short latency peak may be caused in part by an artifact introduced by oscillations (described more fully below) in the following manner: if the oscillation happens to be downward at the start of the gravitropic experiment, the beginning of upward gravitropic bending would be delayed by at least one half-period, but if the oscillation happens to be upward at this time, gravitropic bending would

appear to begin with no delay at all. In those cases where the phase of oscillation is neither upward nor downward and in those cases where oscillation is of very low amplitude, no such artifact would be introduced. Thus, it is possible that the sharp peak at minimum latency may be in part caused by confusion with an upward swing in the oscillation.

The gravitropic bending rate is much less affected by the gravity pre-stress treatment. As shown in Table I, the mean gravitropic bending rate is greater for pre-stressed sporangiophores. This result suggests that the gravity pre-stress treatment not only hastens gravireceptor development but strengthens gravireceptor sensitivity as well. It was shown earlier by Dennison (1961) that increasing the stimulus strength by centrifugation also increases the gravitropic bending rate, conceivably by a similar mechanism.

Almost all of our gravitropic experimental records show oscillation of a 35–55-min period. When gravitropic bending is slow, or during the latency period, this oscillation is clearly apparent as regular cyclic variations in angle with time (Fig. 3, *a*, *b*, and *d*). During rapid gravitropic bending (Fig. 3*c*), these oscillations are more difficult to distinguish but are usually present. In the bilateral rotator experiments, the sporangiophore is adapted vertically and then tipped over horizontally without any change in the (rotating) bilateral blue illumination. In the resulting records, oscillations are seen immediately and do not seem to be in any fixed-phase relationship to the amount of gravity change. These characteristics suggest a circumnutation model with an endogenous oscillator not closely coupled to the orientation of the gravitropic organ (see discussion of circumnutation models in Johnsson, 1979). In the tropostat experiments, which originate in photogeotropic equilibrium, these oscillations can reasonably be explained by the well-known tendency of a sporangiophore to hunt back and forth between two stimuli (Dennison, 1959; Varju et al., 1961). This can be visualized as a control system with feedback, in which each of the two stimuli induces a tropic response that overshoots. However, when the light is switched off (solid arrow, Fig. 3*b*), the oscillation is still present during the latent period and the gravitropic bending, though it is perhaps of smaller amplitude. These results suggest the possibility that the system can oscillate by two different mechanisms, one the internal endogenous model and the other the feedback model. In other cases, such an equilibrium is not present (e.g., Fig. 3*d*), but oscillations nevertheless occur. It is unclear whether these oscillations are analogous to the circumnutation seen in the gravitropic organs of higher plants, but *Phycomyces* may be a promising system in which to ask whether the internal oscillator model or feedback model best describes system behavior (Ney and Pilet, 1981; Heathcote, 1982; Johnsson, 1979).

What is the probable structure and function of the gravireceptor? First of all, it is clear that intracellular rearrangements do occur as a result of exposure to altered gravitational orientation. In the plug stage, this rearrangement occurs within a few minutes of horizontal exposure to gravity (Fig. 5) and consists of the transverse movement of a clear mass. Although we are not yet able to provide details as to the constituents of this structure or its significance in the life of the cell, we nevertheless emphasize that this is the first concrete evidence of an

immediate intracellular reorganization in response to a change in gravity. After several hours of horizontal exposure, however, a different kind of rearrangement occurs, one that is distinguished by a long-lasting asymmetry in the protoplasmic sheath, particularly in the growth zone. It is unclear at present whether these two types of rearrangements are directly connected.

It is highly significant that this asymmetry in sheath thickness persists over long periods of time. The appearance of the cell at 290 min (Fig. 7) differs little from that at 230 min, except that it has grown 0.5 mm during the intervening 60 min. During this time, the growing zone and its corresponding cytoplasmic structures, whatever they may turn out to be, have also moved apically by a distance of 0.5 mm. At the same time, spiral growth will have caused the underlying cell wall to rotate by a significant amount, at least 90°. (In one of our photographic series, a marker on the outside of the cell wall rotated at 3 $deg \cdot min^{-1}$ as it moved from 0.25 to 0.4 mm from the sporangium; thus, in 60 min this part of the cell wall would rotate though ~180°.) Since the thicker sheath layer remains at the bottom during the twisting of the cell wall, there must be some kind of tidal motion of the lower protoplasmic sheath with respect

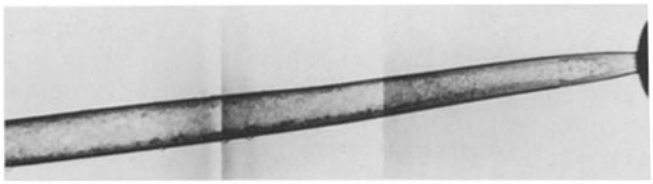

FIGURE 7. Sporangiophore in optical section at 290 min. This cell is the one seen in Figs. 5 and 6. × 112.

to the cell wall. One can visualize the protoplasmic sheath as a lopsided cylindrical toroid, which stays centered in the growing region and whose heavier side remains on the bottom cell wall, even as the latter twists beneath it.

The existence of protoplasmic asymmetry is a plausible first step in the gravireceptor stimulus-response chain. A thicker protoplasmic sheath layer on the lower side could cause a greater cell wall growth rate on that side via several possible mechanisms. For example, a greater mass of protoplasm might be associated with a greater reservoir of cell wall precursor substances. Alternatively, the dimensional asymmetry in the sheath may be related to a differential accumulation of some organelle, perhaps similar to the vesicles described in *Nitella* by Sievers and Schröter (1971). Fine-structure analysis would be needed to confirm this possibility.

The protoplasmic sheath asymmetry could function as a gravireceptor in an entirely different way, however. The integrity and persistence of the asymmetry, in which the thicker part of the sheath continues to stay on the lower wall, even as the latter twists beneath it, requires a continual shearing action, or tidal motion, as the horizontal cell grows and twists. This shearing action might be most prominent in the layer just inside the plasma membrane, and may itself be

an intracellular growth stimulus. The mechanoreceptor property of the sporangiophore has been described by Dennison and Roth (1967); its mechanism has never been worked out, but it could reasonably be based on a shearing action between a semi-rigid protoplasmic mass (the sheath in the growing zone) and some type of responsive structure inside the plasma membrane. If such a shearing mechanism is involved in the gravireceptor mechanism, it should be possible to test the role of cell twist by introducing a counter-rotation, analogous to the back-rotation used by Dennison and Foster (1977) to show that cell rotation is involved in phototropism.

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