Phycomyces: Fine Structure Analysis of the Growing Zone

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ABSTRACT

Fine structure analysis of the stage IVb Phycomyces sporangiophore growing zone (GZ) was performed during steady-state growth using a computer-video digitizer and recorder. By simultaneously measuring the trajectory of two independent particles above and within the GZ, we have confirmed the previous findings of R. Cohen and M. Delbrück (1958 J Cell Comp Physiol 52: 361–388) that the GZ is not uniform. We have been unable to confirm their findings that counterclockwise rotation exists in a mature sporangiophore. The rates of rotation and elongation change independently as a function of position in the GZ. This change is not linear as would be expected if the GZ were uniform. The importance of this finding is discussed in terms of the fibril reorientation model.

The discovery of spiral growth in mature Phycomyces sporangiophores was first made by A. J. Oort (14). Operationally, spiral growth is said to occur if a marker on the GZ is displaced both vertically by elongation occurring below the marker and horizontally because of the rotation of the GZ also below the marker. There is no effect on growth or rotation from the growing zone above the marker; only below the marker. The rotational displacement can be measured either in respect to some fixed object on the sporangiophore, such as the sporangium or to a fixed observer, i.e., the ground. This growth is two-dimensional in the sense that the two-vector components of growth, twist, and stretch, can be thought of as occurring on a plane that has been folded into a conical stalk. Although an early report by Castle (2) suggested that rotation and elongation (twist and stretch) were proportional to one another, recent reports (4, 5, 15) have clearly shown that they are not. Cohen and Delbrück (5) compared the GZ to the flame of a candle based on the fact that any one material section in the GZ is not constant in its behavior but twists and stretches to varying degrees as it moves down the GZ. We have carried this analogy even further by showing that when measured on a minute-to-minute scale, both stretch and twist show independently highly irregular growth velocities similar to a 'flickering candle' (9).

In this report we describe the results obtained from the two-dimensional movement of a single particle as it descends the entire GZ. The movement of this descending particle is compared, minute to minute, with a control particle (stationary with respect to the sporangium) located immediately beneath the sporangium. We have determined that during steady state growth, the upper 5 to 10% of the GZ shows very low rates of twist and stretch. The rates of both rotation and elongation increase dramatically at a definite location in the GZ. The increase in rotational rate starts slower than the increase in elongational rate and decreases faster. Most of the change in rotational rate thus occurs in a small region of the GZ while the change in elongation occurs over a larger section.

MATERIALS AND METHODS

Wild-type Phycomyces blakesleeanus sporangiophores, NRRL 1555(–), originally obtained from M. Delbrück, were grown in shell vials containing 5.0% potato dextrose agar (PDA) with 1.0% yeast extract. The shell vials were incubated under diffuse incandescent light in a high humidity room with a temperature range between 22 and 27°C. Before each experiment, the sporangiophores were dark adapted in red light for at least 20 min. During the experiment, the humidity was kept above 90% RH by surrounding the experimental sporangiophore with open water dishes. The humidity was not measured during the experiment; however, an identical set up was measured by using a General Eastern humidity probe. Unless otherwise stated, all experiments were carried out with a water-filtered red light source.

The apparatus used to simultaneously measure minute-by-minute the net rotation and the net elongation of a stage IVb GZ was first described by Cohen and Delbrück (5) and then modified by Gamow and Bottger (9). The mature stage IVb sporangiophore in a glass shell vial was firmly secured to a stage that rotated clockwise once every 60 s. To ensure that the GZ of the sporangiophore was vertical (parallel to the axis of rotation of the stage), a double knee was inserted between the stage and the vial. The rotating stage allowed us to measure the angular velocity of any particle situated above, below, or in the GZ. Because the net rotation of a mature stage IVb sporangiophore is in the same direction as the rotating stage (clockwise), a particle either above or in the GZ takes less than 60 s to complete one revolution. By determining how much less, we can calculate the angular velocity of a particle placed anywhere on the stalk. This rotation is in respect to the observer. For instance, if a particle completes one revolution in 58 s, we can easily calculate that the entire region below the particle must have a total angular velocity of (60–58)/58 • 360° = 12.41°/min. For our present experiments, we used a glass bead approximately 15 μm in diameter. The bead was placed about 50 μm below the sporangium in the stalk region which does not stretch or rotate (5). The GZ, with the attached glass bead, was observed and recorded on video tape through a camera attached to a microscope. The magnification of the microscope was ×100. An electric timer with a digital printout was used to measure the time with a resolution of 10 ms.

By measuring the angular velocity of a nontwisting particle, a particle located below the GZ, we have determined that our angular velocity measurements are correct to within ±1.7°/min (9). Five data points were taken every 5 min and then averaged, yielding a standard deviation of ±0.8°/min.

A computer-video digitizer was used to measure the rate of elongation. The video signal from the video tapes was sent to a Tecmar video digitizer connected to a Texas Instruments Profes...
sional Computer. The digitizer generates a marker that is superimposed on the sporangiophore which appears on the TV monitor. The computer generated marker can be moved across the monitor with commands entered from the computer's keyboard. It was determined that the marker moves across the image of the sporangiophore in steps of 8.2 \( \mu m \) (i.e. the marker could move across an 82 \( \mu m \) diameter stalk in 10 steps). The glass bead attached to the sporangiophore can be followed with the marker to give an accurate measurement of its trajectory. The computer records the position of the marker. The time is measured using a Panasonic Auto Search Controller which measures the running time of the video tape with a resolution of 1.0 s. This time is input into the computer which calculates the rate of elongation using the time and the position of the marker.

The video digitizer setup provides the only accurate method to measure elongation from the video tapes. Because of the curvature of the video monitor, direct measurements from the screen using a micrometer give a large error. An additional error occurs because the image on the monitor is approximately 5 mm behind the surface of the screen creating a significant error due to parallax. The marker created by the digitizer is not subject to parallax or curvature error because it appears directly on the image of the sporangiophore. The position of the marker is always referenced to the left side of the screen. This ensures that measurement errors do not compound but instead cancel when averaged. By making repeated measurements of a known distance it was determined that a single measurement is accurate to \( \pm 8 \mu m \). One measurement was made every minute and averaged with the measurements from the previous 2 min and the following 2 min. This average measurement of elongation has a precision of \( \pm 2.0 \mu m/min \).

A TV video camera in conjunction with a TV monitor (Fig. 1) has an enormous advantage over the conventional 35 mm photography used previously (9). First, since an entire experiment can be stored on a video cassette and thus become part of a permanent Phycocymes library, we can rerun any given experiment, many lasting several hours, at any future date. Second, since the video tape can be rerun as often as desired, we can simultaneously follow both the rotation and elongation of many particles located on a single GZ. In our present set of experiments, we have measured both the angular and vertical velocities, minute by minute, for 4 h of a single particle located immediately beneath the sporangiophore. Since this region neither rotates nor elongates (either twists nor stretches) with respect to the ground, it serves as an ideal control for our experiments in which we trace the trajectory of a second particle, the test particle. This particle is placed on the very upper edge of the GZ at the beginning of each experiment. As this experimental particle descends the GZ, as a result of stretch, we measured its position, minute by minute, and compared its trajectory to our control particle. The experiments were run for 120 min. A single particle was followed for 115 min during which time it descended from 100 to 200 \( \mu m \). The tape was then rewound and a second particle was followed. The second particle was selected so that it started at 200 \( \mu m \) below the head—just where the first particle had stopped. It was necessary to use two particles because a single particle would take much longer than 2 h. A sporangiophore could not be kept growing straight for more than about 2 h which limited the total time of the experiment. Without the upper control particle, we would not be able to determine whether the experimental particle was slowing down or speeding up because it was passing through the GZ, or because the entire GZ is either increasing or decreasing in its twist and stretch rate.

RESULTS

On the TV monitor screen shown in Figure 1, one can see a mature stage IVb sporangiophore, sporangium, and upper part of the GZ, with several attached glass beads. Figure 1 is included in order to document the high optical resolution obtained from both the sporangiophore and the attached glass beads. Figure 2A shows a typical experiment in which we measured the angular velocity of both a particle attached directly beneath the sporangium (upper curve) and the angular velocity of a second particle (lower curve) which was attached to the upper region of the GZ. The angular velocities of both particles are plotted as functions of time. Below the time axis is an axis which represents the distance from the head of the sporangium to the particle. The three lines between the two axes give a correlation between the scales for three important regions. The distances on the lower axis are given in \( \mu m \) from the sporangium. Because there is so little GZ available for growth or rotation above the particle, the time scale is greatly expanded in the upper GZ (first 300 \( \mu m \)). As the particle descends down the GZ, leaving more and more GZ above, the rate of descent naturally increases. The control particle represents the sum of all the rotation occurring below it. It is the total rate of rotation.

Figure 2B is the same as 2A except the growth rates of the two particles are plotted instead of the angular velocities. The control particle here reflects the overall growth rate of the sporangiophore. The double time-distance axis is the same as used to represent angular velocities.

Figure 3 represents both the rotation and elongation rates as a function of location in the GZ for the same representative experiment. Both curves are estimated best fits through the data points. A straight line cannot be fitted through either set of data without an error much larger than the error inherent in the measurements. A good linear fit would be required if the GZ were uniform throughout. The maximum slope, or maximum rate of change, of the elongation curve occurs around 700 \( \mu m \) below the sporangium. The maximum slope of the rotational curve occurs around 650 \( \mu m \). Most of the change in rotational rate occurs in a section from 400 to 900 \( \mu m \) below the sporangium. This is evident from the steep slope of the rotational curve in this region. The elongation, in contrast, changes much more consistently and continues to change past 1500 \( \mu m \).

Subzone I. That region of the stalk extending from immediately below the sporangium to about 100 \( \mu m \) below it. Subzone I shows no stretch or twist, i.e. a particle placed in subzone I does not change its relative position with respect to the sporangium. This is also the region that has been mechanically determined to be the softest section (8).

Subzone II. This zone constitutes the vast majority of the growing (stretching and twisting) region of the GZ. Twist always occurs here in a clockwise direction and it rarely extends more than 2000 \( \mu m \) below the sporangium. In the upper section of zone II (230 to 600 \( \mu m \)) the rate of change of both elongation and rotation is monotonically increasing. In the lower section the rate of change is monotonically decreasing. This is represented in Figure 3 by the inflection points in both curves around 650 \( \mu m \), which represents the location of maximum growth and rotation.

Subzone III. This zone comprises the rest of the stalk. It is not part of the GZ. Subzone III neither twists nor stretches. It is mechanically quite stiff, showing no extensibility when loaded up to 500 mg using the Instron technique (1). This zone appears to serve little if any metabolic support function for the growing and stretching regions found above it (10).

DISCUSSION

In recent years our laboratory has studied the variety of growth patterns in terms of both the magnitude and the direction of stretch and twist that occurs in the stage IVb GZ. We have shown that these patterns not only change as a function of GZ position but also after the living cell is mechanically deformed (7). These
changes in the growth patterns have led to testable molecular models that appear to account for some aspects of the structure, growth, and regulation of the living cell wall (8, 19). All these experiments have yielded data that are consistent with the model of cell wall growth first developed by Roelofsen and Houwink (18) and recently expanded by Gertel and Green (11), called the multinet theory of growth. The multinet theory is also entirely consistent with the fibril reorientation model that was developed to explain the spiral growth of a mature stage IVb sporangiophore (15).

Multinet growth states that the microfibrils are deposited on the inner surface of the cell wall in a transverse manner and then passively reoriented towards the longitudinal direction as a result of cell wall expansion; the driving force of this cell wall expansion is turgor pressure. Gertel and Green (11) working with Nitella have reported that changing the direction of cell wall strain does indeed change the direction of cell wall orientation as predicted by theory, but in no case could they influence initial transverse orientation which results directly from fibril synthesis. The role of turgor pressure in the rate of cell wall extension, although qualitatively related, has presented a problem that has only recently been solved. The problem was that by using uniaxial extension and matching the cell's longitudinal stresses, the rate of extension of isolated cell walls was significantly greater than the normal growth rate (16). When multiaxial extension stress was induced by filling a nongrowing cell with mercury (12), it was found that these cells were much less extensible than uniaxially stressed cells. This result directly follows the work of Probine and Preston (16) who reported that the transverse modulus is many times greater than the longitudinal modulus. Recently, Metraux et al. (13), using a 'growing Nitella' and an imposed multiaxial stress, have concluded that cell wall extension can only occur by the addition of some 'metabolic event'; this metabolic event may be the laying down of a new primary wall. This is consistent with the known behavior of subzone I; although it is known that the cell wall region in this region is the most extensible one of the entire stalk (8, 17), it neither stretches nor twists, presumably because of the absence of some metabolic event. We would expect that this metabolic event first becomes present in subzone II, although at a low rate.

If the entire growing zone was uniform in structure and metabolism it would be expected that the rate of rotation and elongation would vary directly with the location in the GZ. The two curves of Figure 3 are decidedly nonlinear showing that the GZ is nonuniform in structure, metabolism, or both.

Although the rotation and elongation curves of Figure 3 are subject to the error of fitting a curve to the data set, it is clear that the rotation stops higher in the GZ than does the elongation.
Fig. 2. A, In the upper curve (●), the angular velocity of a glass bead attached directly below the sporangium is measured minute by minute. Each point is an average of five consecutive measurements. The lower curve (△) represents then angular velocity of a second particle that was initially attached to the upper portion of the GZ. This curve is averaged in the same manner as the top curve. The variations in the rate of rotation are not experimental error. They are real fluctuations that have been described by Gamow and Bottger (9). In this representative experiment, the upper (control) particle was placed 64 μm below the sporangium; the second particle started 119 μm below the sporangium and descended to 1500 μm in 230 min. At the bottom of each figure a schematic of a mature sporangiophore is shown indicating the various zones of the GZ. A length axis is given below the schematic. The three lines connect the two different scales to show the locations of the edges of the various zones. Figure B, The upper curve (●) represents the growth rate with respect to the ground of the control particle. This particle is placed in the nongrowing region near the sporangium and therefore represents the overall growth rate of the sporangiophore. The lower curve (◇) is the growth rate of a test particle which is moving down the GZ. The growth rates of both particles were measured once every minute and averaged over a period of 5 min. The schematic sporangium and length scale are the same as those in A. The variations in the rate of elongation are not experimental error; they are real fluctuations that have been described by Gamow and Bottger (9).
This indicates that two or more separate growth mechanisms occur simultaneously. In 1974 we proposed (15) that the left-handed spiral growth of stage IVb and the right-handed spiral growth of stage IVa could be explained via a fibril reorientation mechanism and a fibril slippage mechanism, respectively. If we assume a multinet mechanism in which new fibrils are first laid down in a horizontal position and then passively reoriented towards the vertical during growth, we would expect a much larger rotation to extension ratio in the lower region of subzone II than we have experimentally found. The relatively high extension rates in the lower part of the subzone II can be explained if fibril slippage is assumed to occur there. In general, if a fibril angle at a position in the growing zone is assumed, one can deduce from the data given in Figure 3 the relative amounts of fibril reorientation and fibril slippage at that point. A computer analysis based on the growth curves shown in Figure 3 has shown that the fibril slippage plays an important role in Phycomyces cell wall growth, especially in the lower region of subzone II (M. Wold, unpublished data). Wold has shown that in the upper 52% of the GZ the majority of elongation and rotation must occur by fibril reorientation. The lower 48% of the GZ undergoes elongation and rotation via the fibril slippage mechanism.

It would be of great interest to know whether the entire subzone II responds to sensory stimuli. The data needed to answer this question are in conflict; Cohen and Delbrück (5, 6) reported that the upper region of the GZ is devoid of a light response, whereas Castle (3) reported that this region is light sensitive. We have measured the rate of vertical descent of a test particle located in subzone II away from the sporangium, both before and after a saturating light stimulus and, qualitatively, it appears to us that subzone II is light sensitive. The response is small, but at best one must expect a small response, since we are only observing some 5 to 10% of the entire GZ.

Another discrepancy concerns the existence of 'negative twist' in the upper GZ reported by Cohen and Delbrück (5); we have never observed this negative twist in the mature GZ although we have carefully looked for it. If a region of the GZ did indeed show negative twist, then necessarily we would observe an increase in the angular velocity as the test particle descended through this region because the control particle is rotating in the opposite direction.

It is clear that the GZ of Phycomyces is complex; we feel that this complexity may be more of an advantage than a disadvantage in terms of unraveling the molecular architecture of the living cell wall. The advantage stems from the fact that specific molecular mechanisms can be rigorously tested experimentally and then accepted or rejected.

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LITERATURE CITED

1. ABLQUIST CN, RI GAMOW 1973 Phycomyces: mechanical behavior of stage II and stage IV. Plant Physiol 51: 586–587
8. GAMOW RI, B BOTTGER 1980 The extensibility of the cell wall above the growing zone. Phycomyces 4: Publicaciones de la Universidad de Sevilla 4: 42–43
15. ORTEGA JKE, RI GAMOW 1974 The problem of handedness reversal during
the spiral growth of Phycomyces. J Theor Biol 47:317-332
17. ROELOFSEN PA 1950 The origin of spiral growth in Phycomyces sporangiophores. Rec Trav Bot Néerl 42: 73-110
18. ROELOFSEN PA, AL HOUWINK 1953 Architecture of growth of the primary cell wall in some plant hairs and in Phycomyces sporangiophores. Acta Bot Néerl 2: 218-225