Local Metabolic Autonomy in Phycomyces Sporangiophores

R. Igor Gamow and William Goodell
Division of Biology, California Institute of Technology, Pasadena, California 91109

Received June 19, 1968.

Abstract. The degree of dependence of the growing zone of the Phycomyces sporangiophore upon other parts of the stalk was tested by inhibiting glycolysis and oxidative phosphorylation in the stalk below the growing zone. Initially the growing zone is capable of nearly normal growth when the metabolism in the rest of the stalk is inhibited in this way. However, the growing zone appears to become depleted of something normally supplied from below, because after 3 to 4 hr its growth rate slows down and the growth stops much sooner than in normal sporangiophores. The rest of the sporangiophore appears to have a similar degree of local autonomy because isolated sections from below the growing zone can support protoplasmic streaming for 10 to 20 hr.

The sporangiophore of Phycomyces blakesleeanus is an asexual fruiting body composed of a single celled stalk which supports a sporangium containing numerous spores. The stalk has a 2 to 3 mm growing zone just beneath the sporangium and, at the other end, a “foot” which connects the sporangiophore to the mycelium and allows uptake of nutrients and water. Substrates and subcellular particles are moved up and down the stalk in channels at a rate of about 3 μ/sec (11). The stalk has a diameter of about 0.1 mm and can grow to a length of about 10 cm. After the sporangium has matured, the steady state growth rate is about 3 mm/hr. This rate can be temporarily increased by permanent or temporary increases in the intensity of illumination of the growing zone, and can be decreased by decreases in the light intensity (12).

The aim of this paper is primarily to determine whether the growing zone is dependent upon the rest of the sporangiophore for the substrates and energy necessary for the processes of growth such as cell wall and membrane synthesis. The part of the stalk below the growing zone contains significant quantities of mitochondria, nuclei, and other subcellular particles and might supply the growing zone with substrates. If the growing zone is dependent upon substrates from below, it should have control over the rates of this supply so that they can be adjusted to coincide with the varying growth rates. In cells smaller than the sporangiophore of Phycomyces, protoplasmic streaming and diffusion are rapid enough to move substrates throughout the cell in periods comparable to their turnover periods, and thus allow the adjustment of the rates of synthesis of substrates to their rates of use even though the sites of synthesis and use are separated. However, comparison of the rates of synthesis with the rates of streaming and diffusion within the sporangiophore, indicates that the length of the sporangiophore limits such communication to short sections within the stalk. The turnover periods of ATP and oxygen, both indicative of the level of metabolism (8) within the sporangiophore, are respectively 1 sec (13) and 5 sec. [The turnover period for oxygen was calculated from the rate of consumption per sporangiophore which is about 1.0 × 10⁻⁹ moles/min (Goodell, in preparation) and assuming that the concentration of dissolved oxygen in the sporangiophore is the same as in distilled water at 20°: 0.26 μmoles/ml (7)]. The time which diffusion takes to move small molecules from one end of a 3 centimeter long sporangiophore to the other can be calculated to be several days on the average, and the time required for streaming to be several hr. [The time required for small molecules to diffuse along the sporangiophore is based on the average diffusion coefficient of small molecules in water at 20° which is about 10⁻⁵ cm²/sec (2)]. This difference between the rates of transport and metabolism indicates that the growing zone might have its own supply of substrates and the machinery required for their metabolism.

It has been shown that the sporangiophore as a whole can grow independently of the mycelium (6). If the sporangiophore is plucked and its base put into water, it will continue to grow at a normal rate for about 15 hr. The experiments reported in this paper indicate that growing zones which have been isolated from the rest of the sporangiophore are capable of nearly normal growth for 3 to 4 hr afterwards.

Materials and Methods

Phycomyces blakesleeanus Burgeff [strain 1555 (—) of the Northern Regional Research Laboratory]
was cultured on potato dextrose agar in plates or shell vials as described by Dennison (5). The experiments were done on stage IV sporangiophores (4) because they grow at a constant rate of 2 to 3 mm per hr for many hr and have the most easily observable streaming. All sporangiophores were plucked when in stage IV and initially 2 to 4 cm long. They were either completely immersed in aqueous solution (14) or a portion of the top of the stalk was left out of the aqueous solution. When the tops of sporangiophores were in air, they were kept in humidity close to 100% by placing them in closed vessels containing water or a dilute aqueous solution.

Anoxia was used as the inhibitor of oxidative phosphorylation, and NaF and iodoacetate to inhibit glycolysis. Anoxia was produced by placing a plucked sporangiohore in a microchamber perfused with a stream of nitrogen, or by putting the sporangiophore into a capillary tube (inside diameter 0.6 to 0.9 mm) containing water. This procedure takes advantage of the slow rate of diffusion of oxygen down the capillary compared to the rate of consumption of oxygen by the sporangiophore. See the Results section.) The effect of chemical inhibitors of glycolysis was checked by immersing plucked sporangiophores in 0.01 M and 0.1 M (pH 7) solutions of the inhibitors. Sporangiophores were immersed in phosphate buffer solution (pH 7, 0.01 M) as a control. To inhibit both glycolysis and respiration, sporangiophores were first immersed in 0.01 M NaF solution for 10 min and then put into the nitrogen chamber or into capillaries filled with 0.01 M NaF solution.

The effect of inhibition of glycolysis and respiration in nearby regions upon growth was determined by putting various portions of the lower part of the sporangiophores into capillary tubes filled with 0.01 M NaF solution. The bases of the capillaries were stuck in plasticine (spread on the bottom of a pan) in order to stand the capillaries up and to prevent movement of the NaF solution through the capillary. The pan was then filled with the same NaF solution until the upper lips of the capillaries were just covered. This arrangement prevented evaporation from the top of the capillaries while assuring concurrent anoxia. The growth rate was determined by measuring every 2 hr the net increase in the length of the sporangiophore. To minimize the physical damage to sporangiophores with only 0.2 mm or less of their stalk out of the capillary, a hair was used to push the sporangiophore down into the capillary. The hair was tied to the base of the sporangiophore and both the sporangiophore and the hair were inserted into the capillary. After positioning the sporangiophore inside the capillary the free end of the hair was cut off.

As a check of the effectiveness of this method, varying fractions of the sporangiophore were physically isolated by pinching a short section shut with a small strip of lead (3 mm × one-half mm × one-fourth mm). The stalk below the pinched section was removed to be certain that the clamping was tight enough to maintain turgor pressure in the upper section of the sporangiophore. The sporangiophore was then stood up by embedding the lead strip in plasticine, and 1 to 4 mm of the new "foot" were immersed in 0.01 M phosphate buffer (pH 7).

Growth was measured with a ruler if the interval between measurements was an hr or more. When the average growth rate over shorter intervals was desired, a 17 power microscope was used. In these experiments, any sporangiophore which had not grown after the first 2 measurements was considered physically damaged and was discarded.

If the effect upon streaming of isolating a section of the sporangiophore was to be observed, the stalk on both sides of a short segment was put into a capillary filled with a 0.01 M NaF solution. Segments were also isolated by pinching off and removing the stalk on both sides. In this case the sections were immersed in distilled water.

**Results**

*Inhibition of Oxidative Phosphorylation and Glycolysis.* Methods of inhibition of glycolysis, oxidative phosphorylation or both were tested for their ability to inhibit streaming or growth in regions where they were applied without affecting nearby regions.

If sporangiophores are put into nitrogen streaming stops within 1.5 min in the growing zone and in a varying fraction of the sporangiophore below the growing zone (where there is rapid cessation of streaming, the times required for the inhibition are given in table I). In sporangiophores shorter than 2.5 cm, streaming usually stops throughout the sporangiophores. In longer sporangiophores the proportion of the stalk in which streaming in nitrogen continues for at least 10 min tends to increase as the length of the sporangiophores increases, as does the rate of this residual streaming, although it is never more than about half the normal rate. The inhibition of streaming can be readily reversed by replacing the nitrogen with air. Streaming can be stopped by putting the sporangiophore in nitrogen and then restarted in air many times with no apparent permanent effects. Inhibition of streaming in nitrogen is not due to dehydration of the sporangiophore because water saturated N₂ gas has the same effect and dry air has no inhibiting effect.

Streaming in nitrogen in the lower regions of most sporangiophores longer than 2.5 cm continues for 30 to 40 min. When the sporangiophores are immersed in inhibitors of glycolysis (0.1 and 0.01 M NaF) for 10 min and then put into nitrogen, streaming stops throughout the entire length of the sporangiophores within 90 sec even though the inhibitors of glycolysis alone have no effect upon streaming for at least 2 hr (see table I).

It can be calculated that the sporangiophores inside capillaries (inside diameter 0.6-0.9 mm) filled
Table 1. Effects on Cytoplasmic Streaming of the
Inhibition of Oxidative Phosphorylation
and Glycolysis

The times required to stop streaming in parts a and b are those for stopping it in the upper part of the sporangiophore, except in the case of 0.1 M NaF where streaming stopped throughout the sporangiophore at approximately the same time. In part c the times given are for stoppage throughout the sporangiophore.

<table>
<thead>
<tr>
<th>Inhibitors and the conc used</th>
<th>Time required to stop streaming</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Of oxidative phosphorylation</td>
<td>30-90 sec</td>
</tr>
<tr>
<td>Nitrogen gas</td>
<td>30-90 sec</td>
</tr>
<tr>
<td>Capillary</td>
<td>10-15 min</td>
</tr>
<tr>
<td>b) Of Glycolysis</td>
<td>2 hr</td>
</tr>
<tr>
<td>0.1 M NaF (pH 7)</td>
<td>2 hr</td>
</tr>
<tr>
<td>0.01 M NaF (pH 7)</td>
<td>more than 24 hr</td>
</tr>
<tr>
<td>0.1 M iodoacetate</td>
<td>about 2 hr</td>
</tr>
<tr>
<td>c) Of glycolysis and oxidative phosphorylation</td>
<td>30-90 sec</td>
</tr>
<tr>
<td>0.1 M NaF (pH 7) and nitrogen gas</td>
<td>10-15 min</td>
</tr>
<tr>
<td>0.1 M or 0.01 M NaF (pH 7) and capillary</td>
<td>30-90 sec</td>
</tr>
</tbody>
</table>

1 Chemical inhibitors of oxidative phosphorylation also stop streaming rapidly. KCN (0.001 M, 0.01 M both pH 7), dinitrophenol (0.005 M, pH 7), and sodium azide (0.01 M) stopped streaming in the upper portion of the sporangiophore within 5 min. Streaming stopped throughout the length of the sporangiophore within 30 min. In combination with 0.01 M NaF, streaming stopped within 10 to 30 min. However, the mode of action of these inhibitors is uncertain because oxygen uptake continues normally for at least 2 hr when these inhibitors are added to extracellular lysates of sporangiophores.

2 Anoxia is produced inside a capillary because the sporangiophores use oxygen faster than it is replaced by movement of oxygen down the capillary via diffusion. The time required for streaming to stop is essentially that required for the sporangiophore to use up all the O₂.

Inhibitors and the conc used | Time required to stop streaming |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Of oxidative phosphorylation</td>
<td>30-90 sec</td>
</tr>
<tr>
<td>Nitrogen gas</td>
<td>30-90 sec</td>
</tr>
<tr>
<td>Capillary</td>
<td>10-15 min</td>
</tr>
<tr>
<td>b) Of Glycolysis</td>
<td>2 hr</td>
</tr>
<tr>
<td>0.1 M NaF (pH 7)</td>
<td>2 hr</td>
</tr>
<tr>
<td>0.01 M NaF (pH 7)</td>
<td>more than 24 hr</td>
</tr>
<tr>
<td>0.1 M iodoacetate</td>
<td>about 2 hr</td>
</tr>
<tr>
<td>c) Of glycolysis and oxidative phosphorylation</td>
<td>30-90 sec</td>
</tr>
<tr>
<td>0.1 M NaF (pH 7) and nitrogen gas</td>
<td>10-15 min</td>
</tr>
<tr>
<td>0.1 M or 0.01 M NaF (pH 7) and capillary</td>
<td>30-90 sec</td>
</tr>
</tbody>
</table>

1 Chemical inhibitors of oxidative phosphorylation also stop streaming rapidly. KCN (0.001 M, 0.01 M both pH 7), dinitrophenol (0.005 M, pH 7), and sodium azide (0.01 M) stopped streaming in the upper portion of the sporangiophore within 5 min. Streaming stopped throughout the length of the sporangiophore within 30 min. In combination with 0.01 M NaF, streaming stopped within 10 to 30 min. However, the mode of action of these inhibitors is uncertain because oxygen uptake continues normally for at least 2 hr when these inhibitors are added to extracellular lysates of sporangiophores.

2 Anoxia is produced inside a capillary because the sporangiophores use oxygen faster than it is replaced by movement of oxygen down the capillary via diffusion. The time required for streaming to stop is essentially that required for the sporangiophore to use up all the O₂.

with water quickly produce local anoxia because they use up the oxygen dissolved in the water within a few min and longitudinal diffusion of oxygen inside the capillary is too slow to replace it. [The diffusion coefficient of oxygen in water at 18° is 1.98 × 10⁻⁵ cm²/sec (2).] Streaming in short sporangiophores and in the tops of long sporangiophores inside capillaries again shows greater dependence upon oxidative phosphorylation than does streaming in the bases of long sporangiophores (see table 1). Streaming also stops sooner in the bases of long sporangiophores when the capillaries are filled with NaF solution rather than with distilled water. With NaF streaming always stops within 15 min in that part of the sporangiophore inside the capillary (except the top 1.0 to 1.5 mm section just below the upper lip of the capillary), and continues normally outside of the capillary for at least 24 hr. In the top 1.0 to 1.5 mm section of the sporangiophore inside the capillary, streaming continues for the duration of the experi-

ment because of the availability of oxygen via diffusion.

Streaming often stops temporarily for about 1 min after the sporangiophores have been put into nitrogen and resumes 1 or 2 min later. This re-initiation can sometimes also be seen in sporangiophores placed in capillaries. This phenomenon probably can be attributed to the Pasteur effect.

Anoxia also inhibits growth. When the sporangiophores are placed in nitrogen, the growth rates drop to 10% of the normal value within 3 or 4 min. However, this slow rate of growth continues for at least 15 min. If the sporangiophores are returned to aerobic conditions after 10 min, the growth rate increases slowly and is less than one-half normal 30 min later (K. Bergman, personal communication). Thus the streaming appears to be more rapidly and more completely inhibited by anaerobic conditions than is growth.

NaF has no inhibitory effect upon the growth of sporangiophores. Sporangiophores grown with their bases in NaF and with the top 1.5, 4.5, 9.5, and 14.5 mm of the stalk out of the solution show no significant differences in either the rate or duration of growth. They grow as fast and for as long a time as sporangiophores grown in 0.01 M phosphate buffer (pH 7) (fig 1). Sporangiophores also continued to grow for at least 12 hr when their tops are immersed in 0.01 M NaF.

Effects of the Inhibition or Removal of Part of the Sporangiophore on the Metabolism of the Rest of the Sporangiophore. Inhibition of both streaming and growth can be localized within a part of the sporangiophore through the use of capillaries filled with NaF solution. Using this method (see Methods

Fig. 1. Dispensibility of glycolysis for growth. Growth rates of sporangiophores with their bases in 0.01 M NaF (pH 7) (○) and in 0.01 M phosphate buffer (pH 7) (△) were measured. The points given for sporangiophores grown in NaF solution represent the grand averages of sporangiophores which initially had the top 1.5, 4.5, 9.5, and 14.5 mm of their stalk above the NaF solution. A total of 40 sporangiophores was used. The points given for sporangiophores grown in phosphate buffer represent the average growth of 10 sporangiophores which initially had 9.5 mm of their stalk out of the solution. Each point represents the average growth rate during a 2 hr period.
section) the extent to which part of the sporangiophore is dependent upon the metabolism of the rest was tested. As a check on the completeness of the inhibition of metabolism produced by anoxia and NaF, part of the stalk was removed (see Methods section).

Cytoplasmic streaming continues for 10 to 20 hr when a one millimeter section is physically isolated by pinching off the stalk on both sides. The same result is obtained with sections isolated from the rest of the sporangiophore using capillaries filled with NaF solution. A one millimeter section was left outside the capillaries and streaming continued in this section and in the two 1.0 to 1.5 sections on either side inside the capillaries.

We measured the growth of sporangiophores which initially had the top 0.5 mm, 1.5 mm, 2.5 mm, 9.5 mm or 14.5 mm portions of their stalks outside of the capillaries. As can be seen in figures 2 and 5, both the maximal growth rates and the total growth are much reduced when the top 1.5 mm or less of the sporangiophore stalk is left outside of the capillary. The growth rates of sporangiophores having the top 9.5 and 14.5 mm outside are initially as fast as sporangiophores grown in aerobic NaF or in phosphate buffer; however, after 7 hr the growth rate declines more rapidly and stops sooner than in the controls (figs 1 and 2). The sporangiophores with 2.5 and 4.5 mm outside initially have nearly normal maximum growth rates (respectively 80 and 90 % that of the controls), but the growth rates decline more rapidly than is the case with longer sections outside. Thus there is a sharp increase in both the maximal growth rate and the total growth as a greater fraction of the stalk is allowed to function until 2.5 millimeters are outside of the capillary (figs 2 and 5). As the uninhibited fraction is increased beyond this point the maximum growth rate increases only slightly and the total growth changes much less rapidly than before. However, the total growth never reaches that of uninhibited plucked sporangiophores.

Sporangiophores whose lower portions have been physically removed show about the same net growth as sporangiophores put into capillaries with the same portion outside of the capillary. The growth rates are slower but the growth continues for a longer time (fig 3). The best correspondence in total growth between these sporangiophores and those in the capillaries is obtained if it is assumed that the top 1.0 mm of the stalk inside the capillary metabolizes normally and the rest of the stalk inside the capillary has no active effect upon growth (fig 5).

The growth rates described above are averages for 2 hr of growth. To determine whether sporangiophores with 1.5 mm of the stalk above the capillary could initially grow at normal rates, the growth rate was measured at 10 min intervals with a microscope. The rate of growth of these sporangiophores is reduced to 0.7 mm/hr within the first 10 min (fig 4).

For the first 2 hr the growth rate of any sporangiophore partially inside a capillary is slow. This appears to be caused by inserting the sporangiophores into the capillaries, because sporangiophores which are stuck into empty capillaries so that 9.5 mm remain outside also show this slower initial growth rate. However, the growth rates of these sporangiophores later return to normal. Also the growth rates of sporangiophores which initially have part of their growing zones inside empty capillaries (0.5 and 1.5 mm of the stalk above the capillary) return to normal levels within 2 or 3 hr. Figure 4 shows that sporangiophores with 1.5 mm outside grow at 80 % of the normal rate of plucked sporangiophores within 30 min.

The sporangiophores used in these experiments
varied in their initial length from 2 to 4 cm. However, no dependence on initial length was found in the net growth among sporangiophores whose top 9.5 or 14.5 mm were outside the capillaries in spite of the fact that the longer sporangiophores had a significantly greater fraction of their length inside the capillaries.

Discussion

Streaming continues for 10 to 20 hr in isolated 1 mm sections of the sporangiophore stalk. This indicates that the energy sources and other substrates necessary for streaming are contained within each such section in sufficient quantities to permit such streaming to persist over long periods of time.

The metabolic processes directly associated with growth also appear to be almost completely localized within the growing zone. Sporangiophores with the upper 2.5 mm or more of their stalks outside of the capillary have maximal growth rates at least 80% that of uninhibited plucked sporangiophores. However, the growth rate of sporangiophores with 1.5 mm outside the capillary, is reduced to one-third of normal immediately after the sporangiophores are put into the capillary. Sporangiophores with 0.5 mm outside are capable of growth rates only 10% of normal. This indicates that the whole growing zone must be active to enable the sporangiophore to grow at normal rates.

Long-term normal growth depends also upon the active metabolism of the lower part of the sporangiophore. The total growth and the duration of normal growth increase as the uninhibited portion of the stalk is increased even after all of the growing zone is left out of the capillary. Growth for extended periods may depend upon basic substrates which are translocated to the growing zone and used for repair or for cell wall or membrane synthesis.

The conclusions reached above are somewhat ill defined because not all of the metabolism of the portion of the sporangiophore inside the capillary is inhibited. Streaming in the top 1.0 to 1.5 mm of
the stalk inside the capillary indicates a significant level of metabolism in this region and there may be some metabolic processes taking place in the lower regions. However, the best correspondence in net growth between sporangiophores which are put into capillaries and those with their bases physically removed is obtained if it is assumed that the top 1.0 mm of the stalk inside the capillary metabolizes normally and the portion below has no active effect upon growth. This indicates that the capillary technique inhibits all of the metabolism of the stalk inside the capillary except the upper 1.0 mm. This inference is supported by the observation that after streaming stops (10–15 min in the region below the top 1.0–1.5 mm section), there is obvious degeneration of the cytoplasm, and streaming does not reinitiate if the sporangiophore is removed from the capillary. These observations indicate that the top 2.5 to 4.0 mm of the stalk are necessary for normal initial growth rates.

The growth rates of sporangiophores with their bases removed after the stalk was pinched shut are less than those of corresponding sporangiophores in capillaries. The growth rate appears to be decreased because the stalk cannot take up water as effectively as the “foot” and not because of permanent damage to the growing zone. A waxy layer coats the cell wall except in the growing zone (3), and would probably limit the water uptake rate. Even though sporangiophores, which initially have only 4.5 mm of their stalk remaining, are pinched off close to the growing zone, their initial growth rate (for the first 5 hr) is the same as that of sporangiophores with 9.5 mm remaining. The net growth of all of these sporangiophores is also the same as corresponding sporangiophores in capillaries.

The rapid cessation of growth and streaming when the sporangiophores are in nitrogen or inside capillaries indicates that they are highly dependent upon oxidative phosphorylation, as has previously been shown for both the whole thallus (1) and for the spores (16). In spite of this dependence, the sporangiophores can temporarily change from aerobic to anaerobic metabolism (glycolysis), as indicated by the reinitiation of streaming 2 or 3 min after the sporangiophores have been put into nitrogen and by the longer duration of streaming in the lower region of long sporangiophores of inhibitors of glycolysis are not used. The reinitiation of streaming can probably be attributed to the Pasteur effect because it indicates that the rate of glycolysis is limited by respiration under aerobic conditions and that this limitation is lifted when the sporangiophore is placed into anaerobic conditions. This phenomenon was named by Warburg (15) for Pasteur, who first described it in yeast (9) and later in Mucor (10), a genus of fungi closely related to Phycomyces.

Acknowledgments

We thank Steve Beckendorf, Kostia Bergman, Dr. Marko Zalokar, and especially Drs. Max Delbrück, Charles David, and Martin Heisenberg for many helpful discussions concerning this work and for critically reviewing this paper.

Literature Cited