

Short Communication

# Intracellular Recordings from *Phycomyces*<sup>1</sup>

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PHILIP M. GROVES AND R. IGOR GAMOW

Departments of Psychology and Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80302

## ABSTRACT

Intracellular recordings from the giant sporangiophore of *Phycomyces* stage II were obtained. The mean transmembrane potential for 30 observations was  $-119.9$  millivolts (negative inside), and it did not change either as a result of a light stimulus or during dark adaptation. Injected depolarizing and hyperpolarizing step currents and steady currents did not produce any evidence of spike activity. We conclude that light transduction and dark adaptation in *Phycomyces* are not based on alterations of the transmembrane potential.

The sporangiophore of the fungus *Phycomyces* has been appropriately described as "a gigantic single-celled, erect, cylindrical, aerial hypha . . . sensitive to at least four distinct stimuli: light, gravity, stretch, and some unknown stimulus by which it avoids solid objects" (1). The sequence of development of the sporangiophore of *Phycomyces* can be divided into five stages (2, 3). During stage I the sporangiophore grows upward from the mycelium as a simple pointed cylinder. During stage II the tip of the sporangiophore swells and a bright yellow sporangium, filled with cytoplasm, appears. Stage III is defined by a period of quiescence during which the dimensions of the sporangiophore do not change. The final two stages, IVa and IVb, include the darkening of the sporangium, a change in direction of spiral growth, and the ultimate appearance of the approximately 100,000 spores within the sporangium.

The light growth response of the sporangiophore of *Phycomyces*, studied almost exclusively during stage IVb, consists of a transient change in the rate of growth of its cylindrical stalk (1). Johnson and Gamow (4) described an additional response to light, occurring during the developmental stage II. This response consists of a transient increase in rate of growth of the sporangium after a light stimulus, similar in many respects to the increased rate of growth of the mature sporangiophore.

Willard and Delbrück (6) and Mogus and Wolken (5) have recently reported electrical potentials from *Phycomyces*. Willard and Delbrück used glass micropipettes inserted into stage IV sporangiophores, which were submerged in a conductive

medium, and recorded resting potentials ranging from  $-60$  to  $-130$  mv. Exposure to light, however, did not result in consistent alterations in membrane potential. Mogus and Wolken, on the other hand, utilized sporangiophores plucked from their growing medium, crushed at one end, and submerged in electrolyte baths. Recordings were obtained across the baths, in which the intact end was electrically isolated from the crushed end. Although they did not report mean resting membrane potentials or the range of resting potentials that were encountered, values from published figures appear to range from 15 to 55 mv. In addition, they reported the sporadic occurrence of positive-going alterations in membrane potential upon exposure of stage I and stage II *Phycomyces* to fluorescent light, as well as occasional repetitive, negative-going spike activity. In neither of these experiments were there attempts to determine if the organism was capable of growth under conditions in which electrical potentials were recorded.

In the experiments reported here, intracellular recordings were accomplished by penetration of stage II *Phycomyces blakesleeanus* with glass micropipettes under direct visual observation through a microscope. The lengths of stage II sporangiophores were between 1 and 2 cm, with sporangia from 100 to 300  $\mu\text{m}$  in diameter. Pipettes were filled with 3 M KCl, having tip diameters of from 1 to 3  $\mu\text{m}$  and impedances of from 7 to 18 megohms. Intracellular potentials were recorded with reference to an Ag-AgCl wire electrode placed in the agar-electrolyte medium in which *Phycomyces* was grown, or in a 1 M NaCl bathing medium in which *Phycomyces* was partially or totally submerged. In addition, recordings in several cases were taken with reference to an Ag-AgCl wire loop in contact with the growing zone of *Phycomyces* by means of a saline bridge. Recordings were taken both before and after penetration of *Phycomyces* to insure that membrane potential values were not contaminated by junction potentials between the recording electrodes and the electrolytes in which they were immersed. In some instances, intracellular potentials from a single sporangiophore were recorded with the nonpolarizing reference electrode placed in the agar-electrolyte medium, as well as during subsequent partial and total immersion of the sporangiophore and reference electrode in a saline bath. In every case in which the intracellular pipette could be maintained sealed within the sporangiophore, no changes in membrane potential were seen using these different recording methods. Amplification of intracellular potentials was accomplished by means of a Grass P16, high-input impedance amplifier or by means of a unity gain, M4A WPI Electrometer. Potentials were further amplified and displayed on a Tektronix 564 storage oscilloscope with 3A9 or 2A74 plug-in amplifiers. In experiments in which the Ag-AgCl reference electrode and the sporangiophore were immersed in a saline bath, we injected depolarizing and hyperpolarizing step

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currents and steady current through the micropipette in the range of from 0 to 40 nanoamperes with the aid of an automatic bridge balancing circuit (S. K. Sharpless and S. J. Young, personal communication). In our early experiments, we determined that penetration of *Phycomyces*, resulting in visible leakage of cytoplasm around the micropipette, yielded widely variable transmembrane potentials. In many instances, an initial penetration without leakage of cytoplasm yielded a membrane potential well in excess of 100 mv while subsequent leakage dramatically decreased this to far less than the original value. Thus, only data obtained from penetrations in which there was no visible leakage of cytoplasm around the micropipette are reported here. In an independent series of experiments, we determined that *Phycomyces* grows normally for at least 1 hr with a micropipette inserted into the sporangium without leakage of cytoplasm.

A total of 30 recordings was accomplished without visible leakage of cytoplasm from the sporangium. The mean transmembrane potential for these 30 observations was  $-119.9$  mv (negative inside) with a standard deviation of  $\pm 14$ . We were unable to detect any correlation of membrane potential magnitude with the size of the sporangium.

Six experiments were performed to determine if the membrane potential changed with dark adaptation or subsequent stimulation with white light. In four experiments, electrode penetration was accomplished in white light and the sporangio-phore was then allowed a period in total darkness which varied from 7 min to 36 min for different experiments. During this period, the transmembrane potential (which in these experiments was between 92 and 140 mv for different organisms) was monitored and showed no change during dark adaptation in any of the four experiments. Subsequent to this period of dark adaptation, the room lights were turned on again and the transmembrane potential was again observed. In no case was there any detectable change. In two experiments, *Phycomyces* were adapted in red light for periods of 60 min and 120 min, respectively. Electrode penetration was accomplished in red light (by means of red filters on the microscope light) and white light was then turned on (fluorescent light of  $30 \mu\text{w}/\text{cm}^2$  intensity). No change in transmembrane potential was detected. Because

all of these experiments provided conditions which induce the light head response, we tentatively conclude that the transmembrane potential of *Phycomyces* does not change with a light stimulus.

Injected depolarizing and hyperpolarizing step currents and steady current did not produce any evidence of spike activity in *Phycomyces*.

Our estimate of the transmembrane potential of *Phycomyces* is somewhat higher than those reported by Willard and Delbrück (6), perhaps attributable to the fact that we excluded all penetrations in which leakage of cytoplasm could be detected under microscopic observation. Because the method of Mogus and Wolken (5) was dependent upon leakage of cytoplasm from one end of the organism, this could account for the fact that their published transmembrane potential values are extremely low. The lack of any consistent alterations in membrane potential with exposure to light or dark in our experiments, or during injection of depolarizing or hyperpolarizing current suggests that in the normal, intact, growing plant, transduction of light stimulation is not accompanied by changes in the transmembrane potential.

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