The Influence of Gravity and Light on Developmental Polarity of Single Cells of *Ceratopteris richardii* Gametophytes

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The gametophyte generation of *Ceratopteris richardii* is being used in a number of laboratories as a model plant system for developmental studies (Chasan, 1992). It is an especially valuable system for the study of the effect of gravity on plants in that the detection and response to gravity take place in the single-celled spore during its germination. The most visible evidence of the control of *C. richardii* spore polarity by gravity is that the direction of growth of the primary rhizoid, when it emerges, is downward, in line with the vector of gravity (Edwards and Roux, 1994). However, the direction of rhizoid growth is determined prior to its actual emergence through the spore coat. The influence of gravity on this growth polarity is, in fact, determined irreversibly during a “polarity-determination window”—a period that occurs after germination has been initiated by exposure of spores to light, but before the first cellular division. Gravity does not appear to have an independent effect on the later events of prothallus and secondary rhizoid growth in the gametophyte, for the growth of prothallial cells follows the same direction as that of the first prothallial cell, and the growth of secondary rhizoids follows the same direction as that of the primary rhizoid, no matter how the gametophyte is oriented.

The polarity-determination window is specified by a simple assay in which the spore position is changed by 180° at various times after the initiation of germination by light, but before the first cellular division. Gravity does not appear to have an independent effect on the later events of prothallus and secondary rhizoid growth in the gametophyte, for the growth of prothallial cells follows the same direction as that of the first prothallial cell, and the growth of secondary rhizoids follows the same direction as that of the primary rhizoid, no matter how the gametophyte is oriented.

The polarity-determination window is specified by a simple assay in which the spore position is changed by 180° at various times after the initiation of germination by light, but before rhizoid emergence, and then the subsequent direction of rhizoid emergence and growth is recorded (Edwards and Roux, 1994). Turning the spore upside down before the window opens will also change the direction of rhizoid emergence by 180°; the rhizoid will grow downward in accord with its second position of down. But, if the orientation of a spore is changed 180° after the polarity-determination window closes for that individual spore, the primary rhizoid will grow up, in accord with what was down before the orientation change. If the orientation of a population of spores is changed during the polarity-determination window for that population, some of the primary rhizoids grow in the first direction of down, while the others grow in the second direction of down. Thus, the closing of the polarity-determination window for a population of spores can be determined as the time at which the orientation change has no effect on the orientation of primary rhizoid growth; i.e., the direction of rhizoid growth has already been fixed by gravity prior to this time. The exact time of opening this window for a population of spores and the duration of the open state vary from spore lot to spore lot and can be influenced by growth conditions (Edwards, 1996).

The direction of rhizoid growth is not the earliest visible response of germinating spores to gravity. Before the first cellular division, but after the polarity-determination window, the first indication of cell polarity is migration of the nucleus down (with respect to gravity) along the proximal face of the spore (Edwards and Roux, 1994). This downward migration of the nucleus sets up an asymmetric first cell division, determining the developmental fate of the two daughter cells, one giving rise to the primary rhizoid, and the other giving rise to the prothallus. Thus, in dictating the direction of nuclear migration, gravity also dictates the developmental polarity of the spore. When spores are germinated on a clinostat, the direction of nuclear migration is random, as is the direction of rhizoid growth. However, in each case, the direction of nuclear migration predicts the direction of rhizoid emergence and elongation (Edwards, 1996).

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Although nuclear migration is the most obvious nuclear movement, it is not the first nuclear movement. During the polarity-determination window, the nucleus exhibits random excursions centered around the middle of the spore. These excursions can be visualized through the transparent spore coat, and followed in reference to a fixed trilete marking on the spore coat called the laesura (Edwards, 1996). They can be clearly described in plots showing change in nuclear position over time (Fig. 1). Since the nucleus is the only organelle clearly visible through the intact spore coat by light microscopy, the very likely possibility remains that other organelles move during the polarity-determination window in response to gravity as well. However, nuclei possess sufficient mass to contribute significantly to gravity detection (Sack, 1991). Their centered movements prior to their migration may play some role in the sensing of the vector of gravity, for, as described by Mesland (1992), nuclei are typically tethered to the cell periphery by cytoskeletal elements, and these elements could convey tension and compression forces as the nucleus moves in various directions. Both the centered movements of the nucleus and its directional migration exhibit the same periodic variance in speed (Edwards, 1996). The cause for this is not clear at this time, but may indicate that the same kind of molecular motor machinery is driving both movements.

Another environmental gradient used to control the polarity of fern spore development is unilateral light (Raghaven, 1989). Light gradients do affect polarity of C. richardii spores, but initial experiments using total fluences of about 100 µmol/m² of unilateral white light indicate that the influence of light gradients is secondary to that of gravity.

Tests of the effects of agonists and antagonists on the gravity and light responses are impeded by the limited permeability of the thick spore coat. To overcome this problem, prothallus protoplasts may be used as an alternate experimental system for studying processes related to development of cell polarity. A protocol has been developed that consistently yields viable protoplasts that are capable of regeneration, development into fertile gametophytes, and production of sporophytes. Furthermore, the protoplasts follow a path of regeneration in which a primary rhizoid is formed with the first cell division, thus resembling spore germination. As in spores, polarity of regenerating protoplasts is influenced by unidirectional light and gravity (Edwards, 1996).

The gametophyte generation of C. richardii has many advantages that make it ideal for spaceflight studies. Its small size and easy culture mean that large numbers of individuals can be utilized in a single experiment. Since germination is initiated by light, samples can easily be prepared ahead of time, and germination initiated only after launch with its attendant hyper-g and strong vibrational stimuli. In addition, the primary gravity response, fixing the polarity of nuclear migration and rhizoid growth, occurs in a relatively synchronized population of cells relatively soon after light-induced initiation of germination, and it can be visualized easily through the clear spore coat of Ceratopteris with video microscopy equipment already developed and used in previous shuttle experiments (STL-B). The simplicity of these single isolated cells should facilitate discoveries that will be applicable to more complicated systems and thus lead to advances in the study of the effects of gravity and light stimuli on a wide range of organisms.

Figure 1. Path of nuclear migration in a representative spore germinating on a vertically oriented slide. The position of the nucleus was recorded every half hour. The original position of the nucleus is assigned the position 0,0. Its initial movement is restricted to a region near the center of the spore before it starts its migration downward 15 h after exposure to light. All number values are in micrometers.

Literature Cited


