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Commentary

Commentary on cell polarity in plants: Quatrano 1973

Bacterial, fungal, plant, and animal cells all respond to extrinsic signals to establish intrinsic polarities, which are essential for a whole host of subsequent developmental processes. In this month's commentary, I review a set of classic papers authored by Ralph Quatrano and his colleagues (Crayton et al., 1974; Kropf et al., 1989a, 1989b, 1988; Quatrano, 1973; Quatrano and Crayton, 1973; Shaw and Quatrano, 1996; Stevens and Quatrano, 1978), that set the stage for our understanding of how plant cells achieve polarity. These papers, many of which were published in *Developmental Biology* in the 1970′s and 1980′s, provided a valuable counterpoint to similar studies being undertaken in animal systems at the time, and now serve to illustrate some of the overarching commonalities as to how cells can acquire and maintain polarity to assure specific developmental outcomes.

Quatrano used zygotes of the brown alga, *Fucus vesiculosis*, as an experimental system. The ease of obtaining large numbers of synchronously developing *Fucus* zygotes provided an excellent entry point into defining the cellular processes involved in establishing polarity. Extrinsic asymmetric signals, such as the point of sperm entry or unidirectional light, can polarize the zygote; however, polarity is initially labile and only becomes fixed after several hours. By assessing the ability of zygotes to repolarize after treatment with a variety of biochemical inhibitors, Quatrano and colleagues carefully dissected the roles of a number of biological processes in polarity establishment. Importantly, this approach allowed these researchers to distinguish between the formation of a polar axis and the differentiation of specific cell types.

This strategy was explicitly laid out in Quatrano's paper (Quatrano, 1973), reprinted in this issue, where he showed that the actin cytoskeleton was crucial to the establishment of polarity. In subsequent studies using the same approach, Quatrano and colleagues showed that both Golgi-mediated secretion and an intact cell wall were necessary for normal polarization to occur. These and other investigations led to a model in which local alterations in Ca^{++} flux result in a reorganization of the actin cytoskeleton which is stabilized via transmembrane protein linkages to the cell wall. In turn, this directs the polarized secretion of Golgi-derived vesicles. This targeted secretion serves in part to orient the plane of the first cell division, since plant cells divide through the laying down of a new cell wall between incipient daughter cells. As a result of this oriented division, the daughter cells differ in their size, in their cytoplasmic constituents, and even in their cell wall composition.

These early studies in *Fucus* set the stage for investigating polarity in a variety of plant cells and tissues, with many key transcriptional control mechanisms and signaling components required for polarized growth now being defined in Arabidopsis and other genetically tractable systems. Nonetheless, the studies with *Fucus* arguably remain the best example of our understanding of how specific cellular machineries operate in establishing polarity in plants. Furthermore, the processes by which plant and animal cells establish polarity bear some similarities. In animals, the actin cytoskeleton is linked to the extracellular matrix via integrins to establish cell polarization, critical for asymmetric divisions and directed cell migration. While many of the molecular players are distinct, nature seems to have settled on a common mechanism to 'tie' cytoskeletal asymmetries to the cell exterior to promote a wide variety of developmental outcomes.

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Separation of Processes Associated with Differentiation of Two-Celled *Fucus* Embryos[1]

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Various inhibitors were used to separate the overlapping processes of polar axis fixation, intracellular localizations forming a polar cell, and cell division, all of which are essential for cellular differentiation in two-celled embryos of *Fucus distichus* L. Powell. Cycloheximide and sucrose delayed the appearance of a polar cell (rhizoid formation) without inhibiting the fixation of a polar axis. Cytochalasin B, at 10 μg/ml, reversibly inhibited rhizoid formation without altering cell division. At higher concentrations (50–100 μg/ml) given in short pulses, cytochalasin affected the orientation and delayed the fixation of a light-induced polar axis with no qualitative effect on cell division. Disruption of the mitotic apparatus and prevention of cell division by colchicine had no influence on rhizoid formation or on the photopolarization of the developmental axis.

Unique macromolecules and particles are accumulated and localized in eggs of the angiosperm *Capsella* (Schulz and Jensen, 1968) and in many invertebrates and amphibians (Davidson, 1968). Extensive literature is available to document that this specific polarity, expressed during oogenesis, is of critical importance during subsequent embryogenesis in these animals (Davidson, 1968). Many plant cells undergoing differentiation also exhibit a polar accumulation of substances which are later partitioned into one of the daughter cells (Bünning, 1953; Sinnott, 1960). However, fundamental biochemical and cytological knowledge concerning the initiation, manifestation, and stabilization of such polar cells is lacking and difficult to approach experimentally.

Eggs of the brown alga *Fucus* are visibly apolar and do not undergo polar axis formation or accumulation of specific materials into discrete regions until rhizoid formation 12–14 hr after fertilization (Whitaker, 1940; Jaffe, 1968). The development of this intracellular polarity is the basis of differentiation in the two-celled embryos. Many external gradients, such as unilateral light, can influence the site of these localizations (cf. Jaffe, 1968) and determine the polar axis up to 8 hr *before* the actual appearance of the rhizoid. Using *Fucus* zygotes, then, one can initiate and control events involved with polar axis determination prior to the actual visualization of the localization. However, this polarity is labile until 1–3 hr before the appearance of the rhizoid, at which time an irreversible fixation of the future site of rhizoid formation occurs. Unfortunately, the synchrony of the *Fucus* system does not allow the clear separation of the processes linked to the fixation of the rhizoid site from those associated with the actual formation of the rhizoid (Murphy *et al.*, 1970; Quatrano, 1972) and cell division.

Torrey and Galun (1970) successfully separated rhizoid formation from cell division in *F. vesiculosus*. They demonstrated that zygotes grown in a seawater–sucrose medium and unilaterally illuminated, formed spherical, multicellular embryos. When the apolar embryos were removed from the hypertonic medium, rhizoids formed only from the shaded hemisphere. Since unilateral light was applied from

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only one direction and continuously during this period, it is not known whether a stable polar axis was present in the zygote and passed on to selected cells of the spherical embryo, or whether the polarity was irreversibly fixed in embryonic cells of the shaded hemisphere after immersion in normal seawater just before or concurrent with the emergence of the rhizoids. In either case, however, only a portion of the cell population possessed an internal fixed polar axis (hence, biochemical characteristics of the fixation processes may be masked), and its separatedness from cell division was not demonstrated.

The aim of this study was to separate the fixation of a stable polar axis from rhizoid formation and cell division in an entire population of synchronously developing zygotes, so that each can be biochemically and cytologically analyzed. In addition, the possible role of microtubules and microfilaments in the orientation and stabilization of the polar axis induced by light was investigated through use of the inhibitors colchicine and cytochalasin B, respectively.

Previous results with *F. distichus* demonstrated that: (i) zygotes are maximally photopolarizable between 7 and 11 hr after fertilization, (ii) the site of rhizoid formation is irreversibly fixed or determined at 12–16, and (iii) the actual formation of the rhizoid occurs 14–18 hr after fertilization (Quatrano, 1972). In this study, unilateral light was administered to dark-grown cultures from 7 to 9 hr (LT I). Half of this light-oriented population of zygotes was treated with a reversible inhibitor of rhizoid formation, either cycloheximide (Quatrano, 1968a), or sucrose (Torrey and Galun, 1970). When more than 25% of the zygotes in the other half of the population (untreated controls) formed rhizoids, the inhibitor was washed out of the treated cultures (no rhizoids were observed) and replaced with fresh seawater. A second light pulse (LT II), 90° from LT I, was then given to both treated and control cultures from 15 to 17 hr to determine whether the polar axis was irreversibly fixed. At 48 hr the percentage of rhizoids oriented by LT I and LT II was determined. If the reversible inhibitor of rhizoid formation did not block the fixation of the first light-induced polarity, all rhizoids should be oriented with respect to LT I and their formation separated in time from axis fixation. If LT II influenced the site of rhizoid formation, the inhibitor would appear to prevent events associated with the fixation of a stable polar axis as well, and hence fixation of the axis and rhizoid formation would not be separated.

Since LT II had no effect in orienting the polar axis in the presence of cycloheximide and sucrose (Table 1), it was concluded that neither had any effect on the fixation of an irreversible site of rhizoid formation. However, since rhizoid formation was delayed by 10 hr in these cultures, an entire single cell population underwent rhizoid site fixation 4–6 hr before any zygote in the population exhibited rhizoids. This is also evident in Fig. 1, which demonstrates the time course of the separation of polar axis fixation from the manifestation of the polarity in cycloheximide-treated populations. Since cycloheximide at the concentration employed in this study inhibits protein synthesis by 90% (Quatrano, 1968a) and sucrose, like hypertonic seawater, mannitol, and other sugars, appears to prevent rhizoid formation by a nonspecific osmotic inhibition (Torrey and Galun, 1970), both water uptake and protein synthesis appeared not to be required for polar axis fixation, but essential for rhizoid formation. More importantly, these compounds allowed a clear separation in time between the irreversible fixing of the polar axis from events associated with the appearance of a polar cell in an entire population of single cells.

These results with sucrose would predict that the spherical, multicellular em-

TABLE 1
THE EFFECT OF DIFFERENT INHIBITORS ON PHOTOPOLARIZATION IN *Fucus distichus* ZYGOTES

Treatment[a]	Time inhibitor added (hr)	Experiment	Oriented LT I[b]	Oriented LT II[b]	No. of Cells per Embryo
None	—	A	90.5	9.5	4-6
		B	92.1	7.9	4-6
Cycloheximide[c]	9-15	A	85.5	14.5	3-5
		B	88.0	12.0	3-5
Sucrose[c]	9-15	A	88.9	11.1	3-5
		B	88.2	11.8	3-5
Colchicine[c]	7-17	A	88.5	11.5	1
		B	89.8	10.2	1
Cytochalasin B[d]	9-15	A	19.2	80.8	2-4
		B	25.0	75.0	2-4

[a] Techniques and methods used for synchronization and photopolarization were described elsewhere (Quatrano, 1972). All treatments were subject to unilateral light from 7-9 hr (LT I) and again at 15-17 hr from a different direction (LT II). The number of rhizoids oriented by each light treatment was determined at 48 hr. Each experiment represents a population of at least 200 zygotes.

[b] Percent of the total population of zygotes that formed rhizoids in the shaded quadrant when exposed to unilateral LT I or LT II.

[c] Cycloheximide, sucrose, and colchicine were purchased from Nutritional Biochemicals Corporation (Cleveland, Ohio) and dissolved in Millipore-filtered seawater to a final concentration of 1 μg/ml, 240 mg/ml, and 1 mg/ml, respectively.

[d] Cytochalasin B (Imperial Chemical Industries Ltd., Macclesfield, Cheshire, England) was stored at 4°C in dimethyl sulfoxide (DMSO). The final concentration of DMSO in cultures was not greater than 1%, which had no effect on photopolarization or rate of development. The concentration of cytochalasin B used in the above experiments was 100 μg/ml of Millipore-filtered seawater.

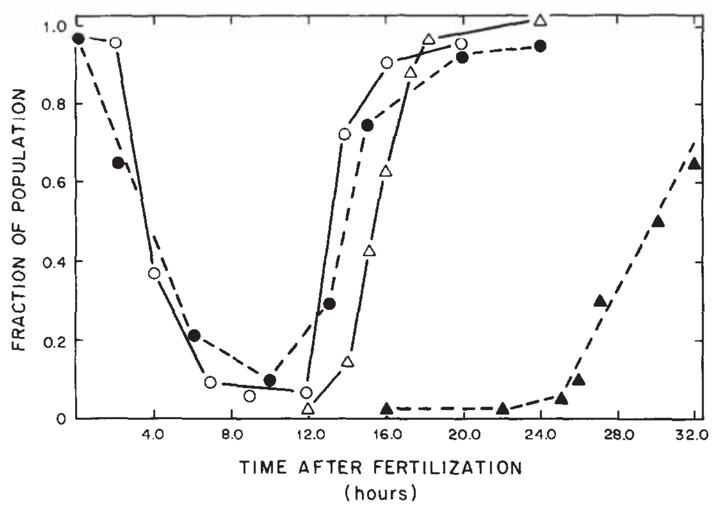

FIG. 1. Time course of sensitivity to polarity-inducing light and the initiation of rhizoid outgrowth in *Fucus distichus*, with (-----) and without (——) cycloheximide treatment (1.0 μg/ml from 7 to 15 hr after fertilization). Each point represents the average of more than 200 zygotes that were exposed to a 2-hr pulse of unilateral light beginning 60 min before and ending 60 min after the designated time. Circles (○) represent fraction of population lacking photosensitivity, and triangles (△) represent fraction of the population possessing rhizoids. Notice how treatment with cycloheximide (-----) effectively separated the fixation of a stable polar axis (●) from the formation of the rhizoid (▲). Similar results were obtained with sucrose.

bryos observed by Torrey and Galun (1970) possessed a stable, fixed polarity long before rhizoid formation in *only* those cells of the shaded hemisphere. It is not known whether these polar cells possessed an accumulation of organelles, vesicles, and/or Toluidine Blue O-stained material at the presumptive site of rhizoid formation, which is indicative of a fixed polar axis (Quatrano, 1972).

During rhizoid initiation and elongation, an elaborate spindle apparatus with astral rays and centrioles is aligned parallel to the polar axis of the cell (Yamanouchi, 1909). To determine whether the orientation of microtubules and/or microfilaments was involved with the initial fixation and subsequent stability of the polar axis, the inhibitors colchicine and cytochalasin B were added to zygote populations at different stages of development.

Colchicine (1 mg/ml), when added at 2–5 hr after fertilization and continually present in cultures grown in diffuse light or total darkness, did not delay rhizoid formation in *F. vesiculosus* (Quatrano, 1968b) or in *F. distichus*. It did not interfere with rhizoid orientation or with the fixation of a polar axis (Table 1) when zygotes were exposed to unilateral light. In all the above cases, cell plates were not present and a clumped metaphase was observed, indicating that the applied colchicine did in fact block spindle fiber (microtubule) assembly. Therefore, colchicine had no effect on the establishment and maintenance of a stable polar axis as well as the initial stages of rhizoid formation. From these data, it appears that cell division was not required for the development of polarity, although no information is now available concerning the role of DNA synthesis.

When cytochalasin B (10 μg/ml) is continuously present in cultures of *F. distichus* zygotes, rhizoid formation is completely prevented but cell division proceeds, yielding multicellular, apolar embryos. Although cell division is not qualitatively affected at these concentrations, the number of cell divisions at 48 hr in treated embryos was reduced compared to controls (an average of 5.4 ± 0.4 cells/embryo in controls compared to 3.2 ± 0.8 cells/embryo in treated). The reversibility of the cytochalasin effect was demonstrated by intermittent treatments (4–6 hr) with the inhibitor which resulted in delays up to 10 hr in rhizoid formation. If cytochalasin (50–100 μg/ml) is present only during a 2-hr unilateral light treatment, the orientation of the rhizoids with respect to the light was markedly altered (52.4% ± 7.3 of the treated population were oriented compared to 97.4% ± 1.9 of control cultures). No significant change in rhizoid orientation was noticed (92.8% ± 6.3 of the treated compared to 95.4% ± 4.8 of controls) if the cytochalasin treatment (9–11 hr) followed the light period (7–9 hr). All these data are similar to results reported by Nelson and Jaffe (1973) using *Pelvetia fastigiata*.

Although cytochalasin did not alter an already established light-induced polar axis, it apparently delayed the period during which the zygote was sensitive to unilateral light. If cytochalasin was present during the period between LT I and LT II (9–15 hr), greater than 75% of the rhizoids at 48 hr were oriented with respect to LT II (Table 1). Therefore, unlike cycloheximide and sucrose, cytochalasin also blocks events associated with the fixation of a stable polar axis. The possible site and mechanism of action of cytochalasin in *Fucus* is unknown. As yet, no contractile-type molecules or structures have been found or directly implicated in rhizoid formation (cf. Wessells *et al.*, 1971; Estensen *et al.*, 1971; Wagner *et al.*, 1972), nor has cytochalasin been shown to interfere with sugar transport (Mizel and Wilson, 1972) or cell membrane–cell surface function (Bluemink, 1971; Sanger and Holtzer, 1972) in *Fucus*. However, use of cytochalasin and the other compounds used in this study to isolate in time the component events of differentiation in two-celled *Fucus* embryos, should aid in approaching the cytological and biochemical basis of these processes.

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