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The role of targeted secretion in the establishment of cell polarity and the orientation of the division plane in *Fucus* zygotes

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SUMMARY

In this study, we investigate the role of polar secretion and the resulting asymmetry in the cell wall in establishing polarity in *Fucus* zygotes. We have utilized brefeldin-A to selectively interrupt secretion of Golgi-derived material into the cell wall as assayed by toluidine blue O staining of sulfated fucoidin. We show that the polar secretion of Golgi-derived material is targeted to a cortical site of the zygote identified by the localization of actin filaments and dihydropyridine receptors. The deposition of Golgi-derived material into the cell wall at this target site is temporally coincident with and required for polar axis fixation. We propose that local secretion of Golgi-derived material into the cell wall transforms the target site into the fixed site of polar growth. We also found that polar secretion of Golgi-derived material at the fixed site is essential for growth and differentiation of the rhizoid, as well as for the proper positioning of the first plane of cell division. We propose that the resulting asymmetry in the cell wall serves as positional information for the underlying cortex to initiate these polar events. Our data supports the hypothesis that cell wall factors in embryos, previously shown to be responsible for induction of rhizoid cell differentiation, are deposited simultaneously with and are responsible for polar axis fixation. Furthermore, the pattern of polar growth is attributable to a positional signal at the fixed site and appears to be independent of the orientation of the first cell division plane. Thus, the establishment of zygotic cell polarity and not the position of the first division plane, is critical for the formation of the initial embryonic pattern in *Fucus*.

Abbreviations: AF, after fertilization; ASW, artificial sea water; BFA, brefeldin-A; F2, highly sulfated fucoidin; FL-DHP, fluorescent-dihydropyridine; GDM, Golgi-derived material; LY, Lucifer Yellow; TBO, Toluidine Blue O; VnF, vitronectin-like protein from *Fucus*; MTOC, microtubule organizing center

Key words: polarity, brefeldin-A, polar secretion, embryogenesis, *Fucus*, cell division

INTRODUCTION

The *Fucus* zygote undergoes a highly asymmetric cell division giving rise to a larger thallus cell and a smaller, polarly growing, rhizoid cell. The thallus cell divides perpendicular to the plane of the first cell division and proceeds in a series of longitudinal and transverse anticlinal divisions to form the body of the *Fucus* embryo. The rhizoid cell grows polarly with each new cell dividing perpendicular to the growing tip. The resulting rhizoid filament eventually forms part of the holdfast structure, which anchors the plant to the substratum. In this way, the first cell division appears to define both the apical/basal polarity of the embryo and the progenitor cells for the plant body and holdfast. In this study, we investigate the role of polar secretion and the resulting asymmetry in the cell wall in establishing polarity and the plane of cell division in *Fucus* zygotes.

The establishment of cell polarity in the *Fucus* zygote is defined by the processes of axis formation, axis fixation and polar (rhizoid) growth. Polar axis formation is characterized by the redistribution of plasma membrane components, including ion channels (Nuccitelli, 1978; see Kropf, 1992), in response to an external vector such as a unilateral light gradient. We have previously shown that a fluorescently labeled dihydropyridine (FL-DHP) binds to a plasma membrane receptor that is polarly translocated to the shaded side of a light gradient in an actin-dependent process (Shaw and Quatrano, 1996). If the light gradient is reversed, these DHP receptors are relocated to the 'new' shaded hemisphere. Actin filaments (F-actin) (Kropf et al., 1989) and a high subcortical calcium ion concentration localize with the DHP-receptors and accurately predict the site of polar growth. We proposed that this cortical region serves as a target site for vesicle secretion at the plasma membrane to support polar growth (Shaw and Quatrano, 1996). Data showing that disruption of either the F-actin cytoskeleton (Brawley and Quatrano, 1979; Brawley and Robinson, 1985) or the internal calcium gradient (Speksnijder et al., 1989) inhibits subsequent polar growth supports this hypothesis.

Polar axis fixation occurs when the labile (formed) axis can no longer be reoriented by changing the direction of the unilateral light vector. We previously showed that polar axis fixation requires F-actin (Quatrano, 1973) as well as the

presence of a cell wall (Kropf et al., 1988). This led to the proposal that a cytoskeleton/plasma membrane/cell wall complex at the site of polar growth effectively immobilizes the asymmetric distribution of ion channels caused by a unilateral light gradient (Kropf et al., 1988; Quatrano, 1990; Goodner and Quatrano, 1993). This model of an axis stabilization complex predicts the presence of transmembrane molecules that may serve to link extracellular factors in the cell wall with cytoskeletal elements, similar to focal adhesions in mammalian cells (Burridge et al., 1988; Jockush et al., 1995) and comparable complexes proposed for budding yeast (Bussey, 1996). The presence of actin strands tightly associated with the plasma membrane/cell wall interface at the site of tip growth in *Pelvetia* supports such a model (Henry et al., 1996).

The formation of the cell wall around *Fucus* zygotes is initially symmetrical and uniform in composition (Quatrano and Stevens, 1976; Quatrano, 1982; Quatrano et al., 1985). Polarized secretion of molecules into the cell wall begins early in development before polar axis fixation or polar growth (Nuccitelli, 1978). A localized jelly secretion, of unknown composition, occurs on the shaded side of the *Pelvetia* zygote in unilateral light. Since this localized secretion can be redirected by changing the position of the light source (Nuccitelli, 1978; Schröter, 1978), it appears to be temporally associated with axis formation. In *Fucus*, polar growth initiates from the site of F-actin (Kropf et al., 1989) and DHP receptor localization (Shaw and Quatrano, 1996) by a process requiring turgor pressure and polar secretion of new cell wall materials (see Quatrano, 1982; Kropf, 1992). The newly secreted cell wall material of the rhizoid includes a protein with vitronectin-like properties (VnF) (Wagner et al., 1992) and a fucoidin (F2), which becomes highly sulfated in the Golgi apparatus prior to secretion (Quatrano and Crayton, 1973; Novotny and Forman, 1974, 1975; Brawley and Quatrano, 1979). However, in *Fucus*, both F2 and VnF are not in the cell wall of the zygote until after axis formation, and then are found exclusively in the cell wall of the rhizoid tip (Wagner et al., 1992).

The resulting asymmetry in the cell wall of the 2-celled embryo also appears to play a role in the differentiation of the rhizoid and thallus cells. Berger et al. (1994) used laser microsurgery techniques to elegantly demonstrate that, in *Fucus* embryos, a rhizoid-derived protoplast redifferentiates in a thallus-like pattern when exposed to cell wall material originating from a thallus cell. Conversely, cell wall material surrounding the rhizoid area will induce a thallus cell to assume a rhizoid-like morphology (Berger et al., 1994; see Fowler and Quatrano, 1995). These data argue strongly for the localization of positional information in the cell wall of the polar, multicellular embryo.

To investigate the role of polar secretion and the resulting asymmetry in the cell wall in establishing polarity, we use brefeldin-A (BFA) to selectively interrupt the secretory process (Miller et al., 1992; Driouich et al., 1993; Staehelin and Moore, 1995) and toluidine blue O (TBO) staining of F2 as a marker (Quatrano and Crayton, 1973) to follow the polar secretion of Golgi-derived material (GDM) into the cell wall of *Fucus* zygotes. We show that the polar secretion of GDM is targeted to a cortical site in the zygote identified by the localization of F-actin and DHP receptors, and that the deposition of GDM into the cell wall at this target site is temporally coincident with polar axis fixation. Our results with BFA indicate that localized secretion is required for polar axis fixation and polar growth, as well as for the proper positioning of the cell division plane and induction of rhizoid cell differentiation.

MATERIALS AND METHODS

Source material (*Fucus disticus* (L.) Powell) was collected and shipped from Yaquina Head, Oregon and stored at 4°C for up to 3 weeks. Synchronous populations of fertilized zygotes were obtained as described by Shaw and Quatrano (1996). The time of fertilization was taken as 15 minutes after submersion in ASW. Cells were allowed to develop in unidirectional fluorescent light (1000 lux) at 16°C. Assays for the determination of polar axis fixation were performed by plating cells on slides in unilateral light at 3 hours after fertilization (AF) and 180° reversal of one slide each hour from 6 to 14 hours AF. The percent of cells with a fixed polar axis was determined by calculating the ratio of cells with rhizoids oriented to the first light direction versus the second light direction at 24 hours AF.

TBO (Sigma) staining was performed by placing zygotes directly into 0.1% TBO in ASW made pH 1.5 with hydrochloric acid. Zygotes were stained for 15 minutes and rinsed in three successive 5 minute washes in 100% ethanol. Stained cells were mounted in tap water and immediately counted or photographed. Vital staining of *Fucus* zygotes with 1.6-5 μM fluorescent-dihydropyridine (4,4-difluoro-5,7-dimethyl-4-boro-3a,4adiaza)-3-(s-indacene) propionic acid (–) DM-BODIPY-DHP (Molecular Probes Eugene, Oregon) (FL-DHP) was performed as described in Shaw and Quatrano (1996). Fluorescein-phalloidin (Sigma) staining for actin was performed by the method of Kropf et al. (1989). BFA was applied to zygotes by diluting appropriate concentrations from a 1 mg/ml ethanol stock directly into ASW. BFA washouts included no less than 3 changes of solution with 5 volumes of ASW. All BFA experiments were performed in unilateral fluor-escent light (1000 lux) in sealed, humidified containers.

FL-DHP and fluorescein-phalloidin were observed using an inverted microscope (Carl Zeiss, Inc., Thornwood, New York), a 40× Neofluor objective lens and an epifluorescence filter set containing a 550±20 nm emission filter. Images were collected using a CCD camera modified for on-chip integration as described by Shaw et al. (1995).

RESULTS

Polarized secretion of GDM coincides temporally with polar axis fixation and predicts the site of polar growth

Polarized secretion of GDM was monitored in a population of *Fucus* zygotes grown in unilateral light by staining F2 with TBO. Deposition of F2 was first observed as asymmetric TBO staining beginning at 8 hours and, by 10 hours AF, half of the cells possessed a localized deposition of F2 in the cell wall of the shaded hemisphere. When polar axis fixation and FL-DHP localization (Shaw and Quatrano, 1996) were monitored in the same population of cells, we found that 50% of the cells had a fixed polar axis by 10 hours AF and 80% an asymmetric distribution of FL-DHP. Hence, the time at which F2 was first observed in the cell wall was temporally coincident with polar axis fixation and several hours after FL-DHP localization. The localization of F2 in the cell wall was correlated in all cases (n>50) with the position of FL-DHP and F-actin (Kropf et al, 1989) at the plasma membrane (Fig. 1A-C). Hence, the localization of DHP receptors and F-actin predicted the site of F2 deposition, which identified the location of subsequent polar growth.

BFA prevents secretion of GDM, polar axis fixation and polar growth

To determine the role of polarized secretion of GDM in the establishment of polarity, zygotes were incubated continually in BFA from either 3 or 7 hours AF. Polar secretion of GDM into the cell wall and polar growth of the rhizoid were completely inhibited in BFA-treated (5 μg/ml) zygotes when observed at 22 hours AF (Fig. 1G). Internal TBO staining was only rarely observed in BFA-treated cells indicating that sulfation of F2 in the Golgi was reduced (data not shown). Zygotes maintained in BFA for several days did not produce an asymmetrical cell as assayed by TBO staining or polar growth. Similar results were obtained with monensin, another inhibitor of secretion in plant cells (Zhang et al., 1993) (data not shown). The effect of BFA was readily reversible, since replacing the BFA media with fresh ASW resulted in a resumption of polar growth, asymmetric TBO staining and sulfation of F2 in >90% of the recovering zygotes.

Since the effects of BFA were reversible and the timing of TBO asymmetry and polar axis fixation were coincident in untreated zygotes, we determined if polar secretion of GDM was required for polar axis fixation. Zygotes grown in unilateral light (light direction 1) were incubated in BFA beginning at 7 hours AF. The BFA was removed at 13 hours AF and the position of unilateral light was reversed (light direction 2). Zygotes that exhibited polar growth (i.e. fixed a polar axis) from the shaded hemisphere of light 1 or light 2 were counted and compared with control cells. For control cells (n=698), 74.5% had rhizoids oriented by light 1, clearly indicating a fixed polar axis by 13 hours AF. For cells treated with BFA (n=673), 76.8% had rhizoids oriented by light 2, indicating that zygotes did not fix a polar axis in the presence of BFA. If BFA was washed out later (at 22 hours AF), and the light orientation reversed, 81% (n=200) of the emerging rhizoids were oriented by light 2. Control cells (n=230) showed a predominant (76%) orientation by light 1. The same results were obtained with BFA-treated *Pelvetia* embryos. Thus, BFA-treated zygotes neither fixed a polar axis nor exhibited TBO asymmetry, but were still capable of initiating polar growth in response to a newly imposed light gradient when BFA was removed after 15 hours (i.e. treatment from 7 to 22 hours AF).

Axis formation, actin localization and cell division are not arrested in BFA-treated zygotes

To determine if actin localization or the actin-dependent redistribution of DHP receptors in the plasma membrane was affected by BFA, zygotes were incubated in BFA and placed in omnidirectional light at 3 hours AF. FL-DHP was added to the media at 4 hours AF and zygotes were placed in unilateral light. DHP receptors were localized on the shaded side of the light gradient in 50% of the zygotes in a population at 8 hours AF, the same time and location as in control cells (Fig. 1A,E). Asymmetric FL-DHP labeling was maintained (but weaker) in zygotes at 22 hours AF (data not shown). BFA-treated zygotes were stained for the presence of F-actin with fluorescein-phalloidin at 10 hours AF. F-actin was concentrated at the cell cortex on the shaded side of the light gradient for >70% of both BFA-treated (n=>50) and control cells (n=>50) that stained with fluorescein-phalloidin (Fig. 1B,F). Hence, BFA treatment had no effect on DHP receptor or F-actin localization to the shaded hemisphere.

Since DHP receptors and actin were colocalized with respect to the light gradient in BFA-treated zygotes, we tested BFA-treated zygotes for the ability to form and maintain a polar axis. Zygotes grown in omnidirectional light were incubated in BFA starting at 6 hours AF and placed in unilateral light at 7 hours AF. At 14 hours AF, the BFA was replaced with fresh ASW and the cells were transferred to darkness for further development. For control cells (n=542), 83% of the population formed a light-oriented, polar axis as indicated by polar growth from the shaded hemisphere of the zygote. For BFA-treated cells (n=396), 51% (expected random probability was 25%) of the cells had rhizoids emerging from the shaded quadrant. BFA-treated zygotes did not exhibit the same degree of adherence to the glass microscope slide as control cells resulting in a displacement of zygotes when BFA containing media was exchanged for fresh ASW. Due to this inherent difficulty in maintaining a fixed orientation of the zygote relative to the light source, the estimated percentage of zygotes with a formed axis is clearly an underestimate. These results indicated that BFA treatment did not disrupt the perception of a light gradient or the ability of the zygotes to maintain the axis in the absence of any orienting vector (i.e. axis formation).

The percentage of zygotes undergoing mitosis was assayed by observing the vital fluorescent dye, lucifer yellow (LY). Zygotes plasmolysed with 0.3 M sucrose in ASW at 1-2 hours AF and then placed in normal ASW containing LY, accumulated the dye in vesicles surrounding the nucleus. The percentage of cells in a population undergoing mitosis was determined by observing the segregation of these vesicles (Fig. 1D). In cells not treated with BFA (n=400), elongation of the vesicle population parallel to the fixed polar axis began soon after rhizoid elongation at 13 hours AF and, by 18 hours AF, half of the zygotes had completed mitosis. For BFA-treated cells, mitosis was completed in 50% of the cells (n=200) between 22-23 hours AF (Fig. 1H), which is approximately 5 hours later than in control cells.

Cytokinesis, determined by the visual appearance of a cytoplasmic partition (Fig. 2A), occurred in half of the zygotes in an untreated population (n=500) at 22 hours AF. Division planes were visible in 10% (n=200) of BFA-treated zygotes by 22 hours AF. By 48 hours, about 70% of the BFA-treated zygotes had formed a single division plane and, occasionally (<10%), a second or third. It appears that preventing polar secretion of GDM by BFA delays but does not inhibit cell division.

The plane of cell division is not oriented perpendicular to the growth axis in BFA-treated zygotes

Division planes that were oriented more than 15° from the perpendicular of the emerging rhizoid occurred less than 2% of the time in untreated embryo populations. Zygotes developing continuously in BFA produced division planes in the spherical embryo (Fig. 3D) that were not oriented perpendicular to the unilateral light gradient (n=250). When zygotes were incubated in BFA from 7 to 14 hours AF about 30% of the resulting embryos exhibited a misoriented division plane relative to the site of polar outgrowth (n=300) (Fig. 3B,C). When BFA was removed later than 22 hours AF, greater than 75% of embryos had misoriented division planes (n=300). The observed division planes were straight and bisected the center of the

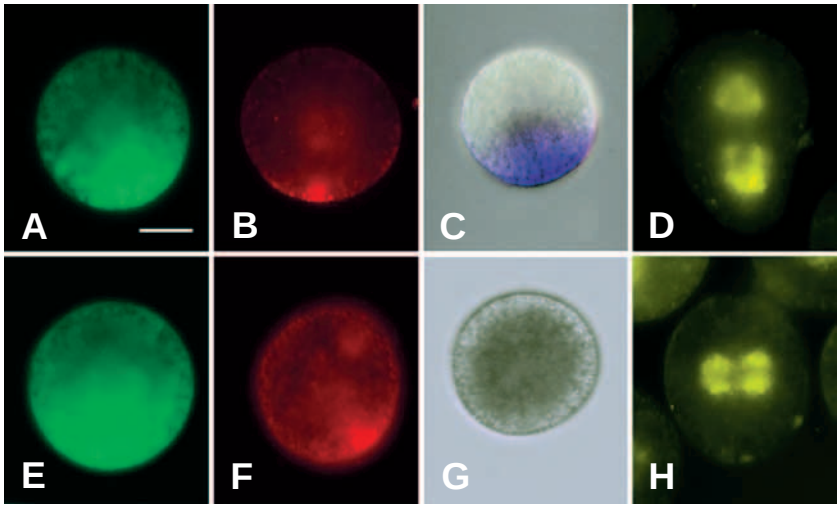

Fig. 1. Treatment with BFA prevents secretion of GDM and polar growth but does not block the development of the zygote. Untreated cells (A-D) and cells given BFA at 6 hours AF (E-H) are placed in unilateral light (from top of figure) at 7 hours AF. Asymmetric FL-DHP labeling of zygotes occurs in untreated (A) and in BFA-treated (E) zygotes at 8 hours AF. Filamentous actin is localized in untreated (B) and BFA-treated (F) zygote at 10 hours AF. TBO staining of F2 is observed in the cell wall of an untreated (C) zygote but not in the cell wall of BFA-treated zygote (G) at 10 hours AF. Lucifer yellow containing vesicles segregate during mitosis in untreated zygote (D) at 16 hours AF and in BFA-treated zygote at 22 hours (H). Bar, 25 μm.

zygote, independent of the orientation to the polar growth axis. Hence, when secretion was inhibited by BFA treatment, the cell division plane did not properly orient either to the unilateral light vector or to the polar growth axis.

When BFA-containing medium was replaced with fresh ASW, secretion resumed and was targeted to the site of F-actin and DHP receptor localization on the shaded hemisphere of zygotes. We observed that polar growth was always preceded by localized secretion of GDM at the target site and that TBO staining was observed exclusively in newly emerging rhizoids. However, we unexpectedly found that the percentage of embryos with two rhizoids (twinned embryos) increased from 1% in untreated populations to nearly 60% in populations that had been treated with BFA from 7 to 22 hours AF. In all cases of twinned embryos, we observed both rhizoids having FL-DHP and TBO staining at their tips and emerging from the shaded hemisphere of the embryo. Furthermore, BFA treatment of embryos with elongating rhizoids stopped polar tip growth. When BFA was removed and tip growth resumed, the growth patterns depicted in Fig. 2 were not observed (data not shown). This is additional evidence that BFA treatment resulting in twinned embryos is due to its effect on a Golgi-mediated secretory process rather than some other effect of BFA itself.

In embryos that had a division plane bisecting the site of the emerging rhizoid (Figs 2C, 4-3), both daughter cells formed rhizoids from the single F2-delimited region defined in the zygote (Figs 2G, 4-3). If cell division occurred before secretion of GDM and polar growth, we inferred that the randomly oriented first division either partitioned the target site into one cell, or bisected the target site into both cells (Figs 2D, 4-4). We base this assertion on the following observations. In 86% of the zygotes that formed twinned embryos after cell division (n=158), the first division plane was parallel with the unilateral light vector and would bisect the region of F-actin and DHP receptor localization (see above) (Figs 2H, 4-4). Zygotes with an initial division plane that was not parallel to the unilateral light vector formed a single rhizoid emerging from the shaded portion of the cell (n=400). Hence, in BFA-treated zygotes, the random division plane either partitioned the target site into one cell, yielding a single rhizoid, or bisected the target site, yielding two rhizoids (Fig. 4-2, 4-3).

Rhizoid and thallus cells differentiate after BFA removal

The further development of embryos recovering from BFA

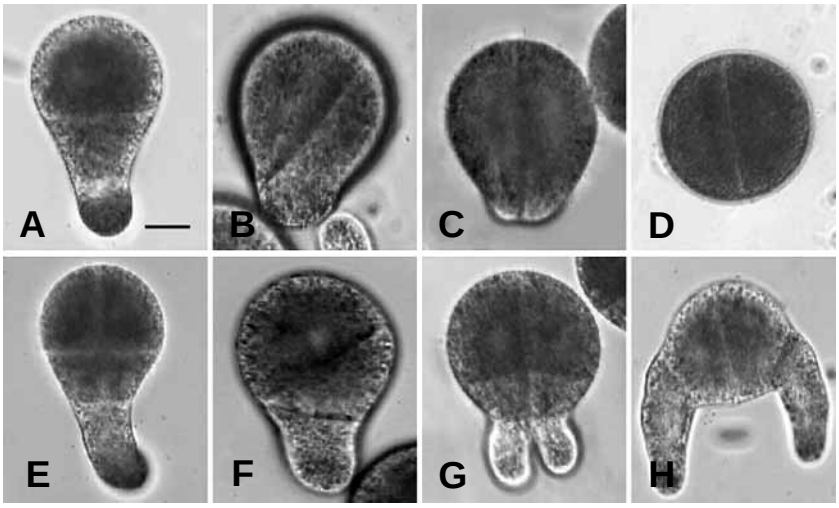

Fig. 2. Polar secretion is required to orient the first division plane perpendicular to the axis of polar growth. The plane of cell division at 22 hours AF in untreated cells (A) is perpendicular to the axis of polar growth. Zygotes treated at 4 hours AF and released from BFA at 12 hours AF (B,C) or at 22 hours AF (D) do not orient the plane of cell division perpendicular to the axis of polar growth as determined by the unilateral light vector (from top of figure). In the untreated embryo (E), the plane of the second cell division occurs in the rhizoid cell parallel to the first division plane, while the third cell division occurs in the spherical thallus cell perpendicular to the first division plane. In embryos recovering from BFA treatment, the second division plane in the rhizoid cell is correctly oriented in the emerging rhizoid (F-H) while the third division, occurring in the thallus cell, orients perpendicularly to the first division plane (G), regardless of its orientation to the polar axis or unilateral light. Refer to Fig. 4 for a schematic diagram of the micrographs pictured in this figure. Bar, 25 μm.

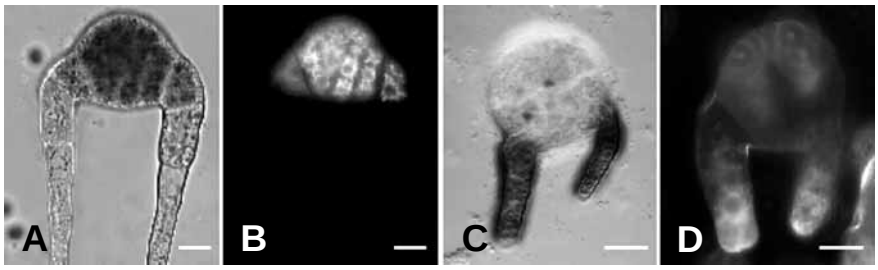

Fig. 3. Rhizoid development in unilateral light is normal in twinned embryos released from BFA treatment (6-22 hours AF). Embryos with two rhizoids (twinned embryos) exhibit normal rhizoid characteristics including polar growth from the shaded side of a unilateral light gradient (from top of figure), vacuolation (A), lack of autofluorescent plastids (B), TBO staining (C), and polar FL-DHP localization (D). Light micrograph (A) and fluorescence micrograph (B) taken of *Fucus* embryo 72 hours AF. Light micrograph of a TBO stained embryo (C) and a fluorescence micrograph of a FL-DHP stained embryo (D) taken at 64 hours AF. Bar, 25 μm.

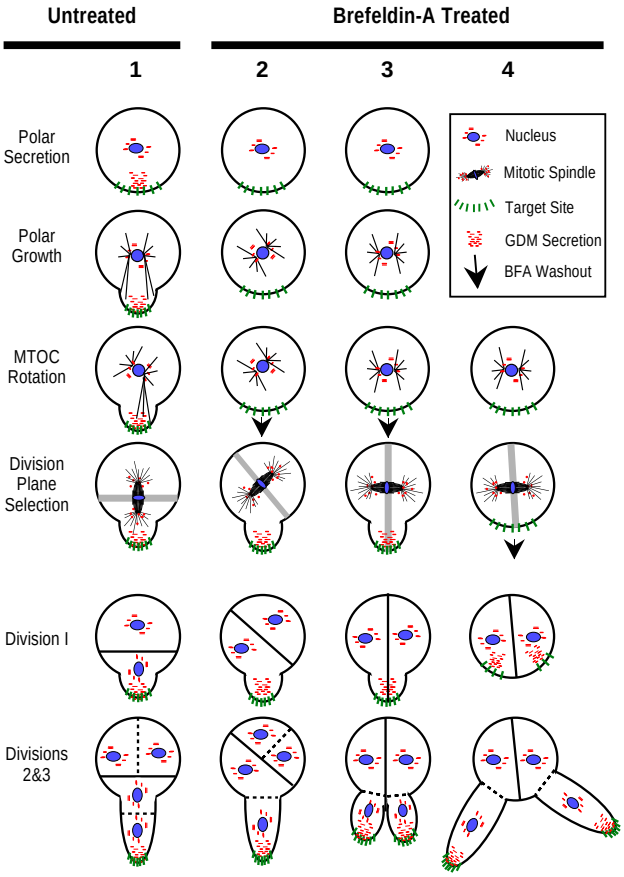

Fig. 4. A schematic model relating polar secretion to the orientation of the polar axis and the first division plane. In the untreated cell (1), polar secretion of GDM to the target site fixes the polar axis relative to an orienting vector (i.e. unilateral light from top of figure) and creates a positional signal at the cell cortex. Polar growth is initiated from this fixed site, followed by the rotation of the microtubule organizing center (MTOC) complex to align the spindle apparatus with the growth axis. Cortical sites are then selected to define the plane of the first cell division. Misoriented division planes in BFA-treated zygotes (2, 3, 4) arise when the positional signal required for MTOC complex rotation (i.e. secretion to the target site) does not occur before spindle construction and the selection of the division plane (2, 3, 4). If the misoriented division plane segregates the fixed site into a single cell (2), only one rhizoid is formed. If the division plane intersects a fixed site (3) or a target site (4), two rhizoids form, but always at the target site (i.e. the shaded side of the light gradient). Subsequent cell division of the thallus cell (dashed line) are perpendicular to the first cell plate, regardless of its orientation to the growth axis, while cell divisions of the rhizoid cell are perpendicular to the growing tip of the rhizoid. Refer to Fig. 2 for corresponding micrographs.

treatment was followed to determine if the resulting thallus and rhizoid cells possessed their characteristic traits (Figs 2, 3). After BFA removal and the resumption of polar secretion of GDM, subsequent cell divisions of the rhizoid were oriented, in most cases, perpendicular to the long axis of the rhizoid (Fig. 2F-H), as in controls (Fig. 2E). Cell divisions in the non-rhizoid cell were oriented perpendicular to the first cell division plane (Fig. 2G), as in controls (Fig. 2E), regardless of the orientation of the first division plane to polar growth. These division planes are shown as dashed lines in Fig. 4. Some exceptions were observed (<10%) when polar growth was slow or absent (data not shown).

Rhizoid-like cells formed after removal of BFA exhibited vacuolation, localized TBO staining (Fig. 3C), FL-DHP labeling (Fig. 3D) and loss of autofluorescent plastids, all characteristic of normal rhizoid cells. Thallus cells in single rhizoid embryos showed none of the above described rhizoid characteristics and, eventually, produced apical hairs (n=35) characteristic of the mature embryo. Twinned embryos produced a normal thallus-like body but did not produce apical hairs after 15 days of development (n=18) (data not shown). Hence, rhizoid and thallus cells differentiated normally in embryos after the removal of BFA and the resumption of secretion of GDM.

DISCUSSION

Polar secretion of GDM is required for polar axis fixation and polar growth

In this study, we have used TBO staining of F2 to monitor polar secretion of GDM into the cell wall. We find that incorporation of GDM into the cell wall occurs at the same time as polar axis fixation and accurately predicts the site of polar growth. At this time, secretion of Golgi vesicles is redirected from a symmetrical to a highly localized pattern and constitutes most of the secretory activity (Quatrano, 1982). We used low concentrations of BFA (Orci et al., 1993; Rothman, 1994) to specifically disrupt this asymmetric deposition of GDM into the cell wall of the nascent rhizoid. BFA treatments of higher plants and budding yeast result in a loss of secretion and an accumulation of Golgi-derived vesicles containing cell wall materials in the cytoplasm (Shah and Klausner, 1993; Driouich et al., 1993; Satiat-Jeunemaitre and Hawes, 1993). Incubation of *Fucus* zygotes in BFA specifically and reversibly reduces sulfation of F2 and polar secretion, as assayed by TBO staining of the cell wall, and blocks polar growth and the ability to fix a polar axis.

Both functional and cytological evidence are presented demonstrating that the effect of BFA is specific to the secretion pathway and not a general inhibitor of cellular development. Mitosis and cytokinesis proceed without defects but are delayed in BFA-treated cells. Most significantly, BFA-treated cells detect a unilateral light gradient, asymmetrically localize both DHP-receptors and F-actin, and form a functional polar axis. Furthermore, if BFA was acting at a site other than at the Golgi, we would have detected vesicle build-up or secretion into the wall with TBO as we did in a previous study using an inhibitor that did not interfer with Golgi function, i.e. cytochalasin B (Brawley and Quatrano, 1979). On the basis of these data, we conclude that the principle effect of BFA on the *Fucus* zygote is a selective disruption of secretion of Golgi-derived secretory vesicles which prevents polar growth and polar axis fixation. BFA appears also to inhibit sulfation of F2 in the Golgi apparatus as judged by the reduction or absence of TBO staining (compare Fig. 1C and G). Further, this work demonstrates that the localized presence of an F-actin patch, DHP-receptors, and the other components of the formed axis are not sufficient to fix the polar axis. We propose that localized F-actin (Kropf et al., 1989), DHP-receptors and the underlying ion gradient (Shaw and Quatrano, 1996), compose a target site for secretion of the material required for fixation of the polar axis.

Only two treatments, in addition to BFA, have been shown to reversibly prevent polar axis fixation in *Fucus* zygotes. Treatment with cytochalasins (B or D), which perturb the actin cytoskeleton, prevents axis formation and fixation without interfering with F2 sulfation (Quatrano, 1973; Kropf et al., 1989) and leads to the accumulation of TBO staining vesicles in the perinuclear region. These F2-containing vesicles are not properly targeted and secreted until the drug is removed (Brawley and Quatrano, 1979; S. L. Shaw, unpublished data). If cytochalasin is added after F2 secretion, the F2 remains in place and the axis remains fixed (S. L. Shaw, unpublished data). Enzymatic removal of the cell wall also prevents axis fixation but not axis formation (Kropf et al., 1988). Thus, all three treatments that prevent polar axis fixation prevent the localized accumulation of F2 into the cell wall either by disrupting targeted secretion or by eliminating the cell wall. Unlike cytochalasin treatment, which prevents assembly of the target site (Shaw and Quatrano, 1996), BFA treatment and cell wall removal (Kropf et al., 1988) do not inhibit axis formation. These data support the hypothesis that polar secretion of GDM is required to stabilize the position of the cortical target site, preventing it from becoming relocated by a light gradient from a different direction. Hence, the GDM polarly secreted before polar growth is not only new cell wall material for growth and adhesion of the rhizoid, but also contains molecules required for polar axis fixation.

Evidence for the role of polar secretion in polar axis fixation extends the axis stabilization complex model as proposed by Quatrano (1990). The model predicts that a cell wall component(s) interacts with the actin cytoskeleton through a putative transmembrane receptor (Fowler and Quatrano, 1995), much like the extracellular glycoproteins fibronectin or vitronectin, and their associated sulfated heparin, bind the RGD peptide receptor in the integrin protein (Burridge, 1988; Jockush et al., 1995). We propose that the polar secretion of molecules sequestered in Golgi-derived vesicles into the cell wall may provide the extracellular ligand(s) for the transmembrane receptor postulated in the axis stabilization complex model. Whether the sulfated fucan F2, VnF or other macromolecules associated with these vesicles correspond to such ligands remains to be determined.

Polar secretion is required to coordinate the position of the cell division plane with polar growth

Allen and Kropf (1992) have shown that the microtubule organizing centers (MTOCs) on opposing sides of the nucleus rotate in *Pelvetia* zygotes, first to a position perpendicular to the emerging rhizoid and then, a second time, to a position aligned parallel with the emerging rhizoid where the spindle is constructed (see Kropf, 1994). The final position of the mitotic spindle appears to then define the plane of cell division (Fig. 4-1). Zygotes continually treated with BFA or released from BFA treatment after 14-20 hours AF show a division plane by 48 hours AF that is not oriented with respect to the polar axis. These results point to an additional role of the positional information normally provided by polarized secretion into the cell wall, i.e. to coordinate the plane of cell division with the polar axis. Apparently, if a positional signal has not been incorporated into the cell wall at a point in the cell cycle just prior to spindle formation (e.g. in BFA-treated zygotes), the MTOC complex is not oriented and cell division occurs in a plane defined by the original position of the MTOCs. Hence, we found that the ability to coordinate the position of the first division plane with polar growth is compromised in zygotes treated with BFA.

The random orientation of division planes in morphologically symmetrical BFA-treated zygotes observed in this study suggests that a formed polar axis with localized F-actin (Kropf et al., 1989) and DHP-receptors (Shaw and Quatrano, 1996) (i.e. the target site), is not sufficient to orient the cell division plane with respect to the unilateral light vector. Previous studies also provide evidence that polar growth per se is not required for correct orientation of cell divisions. Morphologically symmetrical 2-celled embryos formed in hypertonic sucrose media undergo a normal oriented first division, i.e. perpendicular to a unilateral light gradient (Novotny and Forman, 1974; S. L. Shaw, unpublished data). These symmetrical embryos polarly secrete F2 into the cell wall and fix a polar axis prior to cell division (Torrey and Galun, 1970; Quatrano and Crayton, 1973). Hence, axis formation is not sufficient and polar growth is not required for correct positioning of the cell plate. Local incorporation of GDM into the cell wall appears to be the essential signal for coordinating the orientation of the division plane with the polar axis.

Rhizoid growth and differentiation require polar secretion but are independent of the position of the division plane

The development of zygotes released from BFA treatment resulted in the formation of normal, polar 2-celled embryos, even though the first division plane was misoriented. In these cases, the division plane partitioned the fixed site completely into one of the two daughter cells, resulting in a single rhizoid. In over half of the cells recovering from BFA treatment, we unexpectedly observed the formation of embryos with two rhizoids. These twinned embryos, however, always had characteristic rhizoids (i.e. F2 secretion, FL-DHP localization, lack

of autofluorescent plastids, vacuolation, polar growth) emerging from the shaded side of the light gradient. We propose that the increased rate of twinning occurs, in the majority of cases, because the random first division plane bisects either a fixed axis (Figs 2C, 4-3) or the site of a formed axis (e.g. target site) (Figs 2D, 4-4). When a fixed site is bisected, polar growth initiates from both cells at the site of the original F2 deposition into the zygotic cell wall. When the unfixed target site is bisected by a randomly oriented division plane, each half of the target site serves independently as a focus for targeted secretion of GDM, once BFA is removed. Secretion of this material into the cell wall then fixes these two sites, each of which then undergoes polar growth and rhizoid differentiation forming the twinned embryo (Figs 2D,E, 4-4). Both daughter cells in these embryos execute equivalent developmental programs once released from the action of the drug. From these data, we conclude that the cytoskeletal/cell wall complex at the site of F2 deposition directs the differentiation of the rhizoid cell independently of the zygotic division plane and what components of the cytoplasm are initially partitioned into daughter cells. Thus, we argue the establishment of zygotic cell polarity and not the position of the first division plane, is critical for the formation of embryonic pattern in *Fucus.* Since the pattern of cell divisions up to the octant stage in the *Fucus* embryo is nearly identical to that of *Arabidopsis* (Jürgens et al., 1991; Fowler and Quatrano, 1995), observations in this study may be relevant to the *fass* mutation in *Arabidopsis* embryos where the orientation of cell division is abnormal but the apical-basal pattern is maintained (Torres-Ruiz and Jürgens, 1994). In addition, two recent studies by Summers et al. (1996) and Ruffins and Ettensohn (1996) have shown that in the sea urchin *Lytechinus variegatus*, the orientation of the first cleavage furrow does not specify nor is it predictive of the bilateral axis of symmetry.

Our results support in part the conclusion of Berger et al. (1994) that the cell wall derived from the rhizoid portion of 2-celled embryos induces redifferentiation of growing thallus cells into polarly growing rhizoid cells. Implicit in their work is that the distribution of the rhizoid-determining factor in the cell wall is polarized along the developmental axis of the embryo. However, the time at which this information is deposited during development is not clear. Since distinctive characteristics of the rhizoid cell arise in a portion of the zygote long before cell division occurs, we speculate that polar distribution of this factor in the embryo could occur during development of the zygote, more specifically, at the time of BFA-sensitive secretion and the fixation of the polar axis. Our data are consistent with the hypothesis that the factor(s) in the cell walls of embryos responsible for rhizoid differentiation (Berger et al., 1994) are deposited simultaneously with and are a positional signal for polar growth. The role of targeted secretion from the Golgi, resulting in an asymmetry of the macromolecules present in the zygotic cell wall of *Fucus*, now provides an exciting model for the study of extracellular positional information during plant development (Fowler and Quatrano, 1995; Brownlee and Berger, 1995).

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