CYTOPLASMIC ORGANIZATION DURING PATTERN FORMATION IN THE ALGA MICRASTERIAS

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SUMMARY
Using an improved fixation for electron microscopy, we have observed an ordered cytoplasmic organization during pattern formation in the alga Micrasterias. At the earliest stages of morphogenesis (the bulge to 3-lobe stage), as observed previously, electron-dense vesicles and endoplasmic reticulum were found towards the cell periphery, while mitochondria and Golgi material were located internally. In contrast to previous studies, which found no organization in the cytoplasm of growing lobes, we observed a single cluster of electron-dense vesicles at the tip of each lobe at or beyond the 5-lobe stage. In lobes about to branch, two separate clusters of electron-dense vesicles were present in each lobe, apparently foreshadowing the sites of new lobe outgrowth. Individual lobes also possessed an ordered distribution of organelles. Immediately basal to the lobe tip, the concentration of electron-dense vesicles decreased and rough endoplasmic reticulum was more prevalent, followed more deeply in the cytoplasm by increased concentrations of mitochondria and then Golgi material. This cytoplasmic organization in individual lobes is characteristic of tip growth, thus suggesting that individual lobes elongate by this process. We have also investigated the effect of the ionophore A23187 on this cytoplasmic organization. The polarization of rough endoplasmic reticulum, mitochondria and Golgi material was destroyed by ionophore treatment in the presence of 2 mM-MgCl₂ or 2 mM-CaCl₂. The tip-associated clusters of electron-dense vesicles were dispersed by A23187 in 2 mM-CaCl₂ but not significantly affected by A23187 in 2 mM-MgCl₂. The latter observation indicates that the vesicle pattern can be disrupted by high calcium, but not high magnesium concentrations.

INTRODUCTION
Micrasterias is a unicellular green alga that possesses a complex cell wall pattern. The mature cell consists of two symmetrical halves, or semicells. Upon division, each semicell regenerates a mirror image of itself (Fig. 1).

How the pattern of cell wall growth is regulated during morphogenesis in Micrasterias is unknown. Although a number of investigators have sought a structural basis for this process, few clues have emerged. Brower & Giddings (1980) showed that the concentration of particles in freeze-fractured membranes is highest at the tips of expanding lobes, but in electron-microscope studies of the cytoplasm of growing lobes no corresponding cytoplasmic organization has been found. The

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Fig. 1. Cell wall profiles in *M. thomasiana* morphogenesis. Stages are septum, bulge, 3-lobe, 5-lobe, 9-lobe, 17-lobe, 33-lobe and the completion of morphogenesis. The central or polar lobe branches only slightly late in morphogenesis. At later stages the side lobes do not bifurcate synchronously. Mature cells are about 200 μm in diameter.

Arrangement of microtubules appeared unrelated to cell wall pattern formation (Kiermayer, 1968), and the distribution of different vesicle types was reportedly random (Kiermayer, 1970a,b). Subsequent electron-microscope studies have not altered these conclusions (Noguchi, 1976; Ueda & Noguchi, 1976; Menge, 1976; Pihakaski & Kallio, 1978; Brower & Giddings, 1980).

Developing *Micrasterias* are difficult to fix properly for electron microscopy. After standard fixation protocols one type of vesicle bursts and obliterates much of the surrounding cytoplasm (Kiermayer, 1968; Brower & Giddings, 1980). We have used an improved fixation procedure which reduced vesicle swelling. In contrast to the results of previous studies, cells fixed by this method possessed an ordered cytoplasmic organization.

In adequately fixed cells we observed a single cluster of electron-dense vesicles at the tip of each expanding lobe. In lobes about to branch, two separate clusters of these vesicles appeared prior to branch formation. Individual lobes also possessed an ordered distribution of organelles. Basal to the cluster of vesicles at the lobe tip, endoplasmic reticulum, mitochondria and Golgi material appeared sequentially. This organization is characteristic of tip-growing cells, thus supporting the hypothesis that individual *Micrasterias* lobes elongate by this mechanism.

It has been proposed that tip growth is mediated by locally high concentrations of calcium at the growing tip. Using the ionophore A23187, which transports Mn$^{2+}$, Ca$^{2+}$, Mg$^{2+}$ and H$^+$ across membranes (Reed & Lardy, 1972), we found that the tip-associated clusters of electron-dense vesicles could be dispersed by high calcium, but not high magnesium concentrations.
MATERIALS AND METHODS

Culture

*Micrasterias thomasiana* (LB 549) and *Micrasterias rotata* (LB 1941) were obtained from the Algal Culture Collection, University of Texas. No significant difference was observed between these species in our experiments.

Cells were grown in Waris medium (Waris, 1953) with CaCl₂ substituted for CaSO₄. A soil/water extract (Starr, 1964) was added to the medium (≈10% by volume) to enhance growth. The cells were maintained at 19°C on a 12 h/12 h, light/dark, cycle. Dividing cells were induced by reculturing old cultures in fresh medium about 40 h prior to use.

Electron microscopy

Cells were fixed for transmission electron microscopy using a modified form of Brower & Giddings' (1980) protocol. Dividing cells were transferred to a solution of 1% glutaraldehyde, 2 mM-CaCl₂ and 0.05 M-cacodylate buffer kept at 0°C. Thirty seconds later an equal volume of cold 2% OsO₄, 2 mM-CaCl₂ and 0.05 M-cacodylate buffer was added. This mixture was left at 0°C for 1 h. After washing for 15 min in 50 mM-CaCl₂, the cells were transferred to a 2% uranyl acetate solution at 0°C and left for 3 h. Dehydration was through a graded acetone series (30%, 50%, 70%, 80%, 95%, 2×100%; 10 min in each solution) during which the cells were allowed to warm to room temperature.

The following changes in the fixation protocol were tried: (1) varying the concentration of CaCl₂ (2-10 mM) or cacodylate buffer (0-025-0.1 M); (2) fixing at room temperature for 20 min; (3) adding 1-2% sucrose; (4) fixing simultaneously in glutaraldehyde and OsO₄; (5) eliminating the post-fixation in uranyl acetate; (6) fixing in OsO₄ only. These modifications typically led to poorer fixation, although most cells retained remnants of the cytoplasmic organization observed after the standard protocol.

Cells were embedded in Epon. For flat embedding, plastic capsules were used turned upside down. The caps were separated from the capsule and individual cells were transferred in a drop of Epon to the inner side of the cap. Most of the Epon in the drop was pipetted out so that the cell rested flat on the plastic surface. The capsule tip was cut off with a razor blade, and the hollow plastic cylinder that remained was fitted back into the cap. The upside-down capsules were filled with Epon and then the embedding medium was polymerized at 60°C.

Sections were cut on a Porter-Blum MT-2 ultramicrotome and collected on 100 mesh parlodion/carbon-coated grids. The sections were stained in 2% uranyl acetate for 1 h and then 0.2% lead citrate for 2-4 min, or with 5% phosphotungstic acid for 1 h. Sections were examined in a Siemens 101 electron microscope operating at 80 kV.

Quantification of micrographs

The distribution of vesicles and other organelles was quantified using micrographs of 13 individual lobes from cells at the 5- or 9-lobe stage. As discussed above, cells were carefully oriented during embedding. Sections were cut in the radial plane of the cell, and only medial sections of lobes were selected for measurements. Each lobe was magnified from ×15000 to ×21000 as determined from a calibration grid. The magnified lobes were marked off in segments corresponding to 1-5 μm in length drawn perpendicular to the apical-to-basal axis. Seven different kinds of vesicles, in addition to mitochondria and Golgi material, were identified and counted in each segment. The segment outlines were digitized using a Summagraphics bit pad connected to a Commodore 2001 computer, which calculated the area enclosed by the digitized points. The concentration of each vesicle type (or organelle) was found for each segment by dividing the number of vesicles of that type in that segment by the area of the segment. Even though the total number of vesicles of a particular type sometimes varied considerably from one section to the next, their pattern of distribution did not change. Therefore we have normalized the vesicle concentration data by dividing vesicle concentration per unit area in each segment of a lobe by the total number of vesicles of that type in that lobe. This normalization removes major variations that could be encountered due to section thickness, including the Holmes effect (Weibel, 1969).

For cells treated with A23187 a simpler quantification method was employed. Micrographs were magnified to ×8300, and then marked off in segments corresponding to 3 μm in width along the
Fig. 2. Cell at the bulge stage. Vesicles predominate around the periphery, while mitochondria (m) and Golgi material (G) are usually, but not always, found near the nucleus (n). ×5600; bar, 3 μm.

lobe apical-to-basal axis. At several positions within each segment, the number of vesicle profiles in an area of 2-9 μm² was determined. These totals were averaged to estimate the concentration density of vesicles in that segment.

Treatment of cells with A23187

The ionophore A23187 was obtained from Sigma Chemical Co., St Louis, Mo. Stock solutions were made up in ethanol at 5 mg/ml⁻¹ and kept in the dark at −20°C; a concentration of 20 μM-A23187 was routinely used. This yielded a solution of 0.002% ethanol, which in control studies had no effect on the ultrastructure or growth of dividing cells.

To study the effects of A23187 on cell ultrastructure, cells were transferred to either 20 μM-A23187 + 2 mM-CaCl₂ or 20 μM-A23187 + 2 mM-MgCl₂ for 10 or 35 min, and then prepared for electron microscopy.

Fig. 3. A single cluster of electron-dense vesicles is present at the tip of each lobe (filled arrows, A–D) and not at the lobe clefts (open arrows, B, C). A. Lobe from a cell at the 9-lobe stage. The chloroplast (c) with pyrenoid (p) occupies the lobe base. B. Lobe from a cell at the 5-lobe stage. Section stained with phosphotungstic acid. Mitochondria (m) and Golgi material (G) stain weakly, while the electron-dense vesicles stain strongly. C. Lobes from a cell at the 33-lobe stage. Part of a vacuole (v) is evident. D. Polar lobe from a cell at the 5-lobe stage. A, B, ×5320; C, ×3140; D, ×3990; bars, 3 μm.
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RESULTS

Ultrastructure of developing cells

In cells at the early bulge to 3-lobe stage, electron-dense vesicles and rough endoplasmic reticulum were found predominantly towards the cell periphery (Fig. 2). Mitochondria and Golgi material were usually, but not always, located internally near the nucleus.

In cells at all later stages (the 5-lobe to the 33-lobe stage), we observed a single cluster of electron-dense vesicles at the tip of each elongating lobe (Fig. 3). A single cluster of vesicles was also observed at the tip of the polar lobe (Fig. 3D). In lobes about to branch, two separate electron-dense vesicle clusters were observed at the
unbranched tip (Fig. 4). These regions presumably corresponded to the sites of new outgrowth that would have led to branching of these lobes. At later stages of morphogenesis, the polar lobe branches slightly (Fig. 1). Correspondingly, a bilateral distribution of vesicles was typically observed in this lobe by the 16-lobe stage (data not shown).

When sections were stained with 5% phosphotungstic acid (PTA), the electron-dense vesicle clusters at the tips of lobes were prominent (Fig. 3B). PTA primarily stained the cell wall, the cell membrane and the contents of certain of the tip-associated vesicles (Fig. 5A). Golgi bodies, mitochondria and rough endoplasmic reticulum were not markedly stained by PTA, although stainable electron-dense vesicles were associated with Golgi cisternae (Fig. 5B).

In PTA-stained sections, seven different kinds of vesicles were identified (Table 1). Using this classification scheme, we quantified the tip-to-base distribution of vesicles in lobes from semicells at the 5- or 9-lobe stage (Fig. 6). An analysis of

Fig. 5. A. Vesicle types in sections stained with phosphotungstic acid (PTA). Close-up of tip in Fig. 2B. Vesicle nomenclature described in Table 1. PTA also stains the cell wall (also Fig. 2B). The intensity of the wall stain is comparable to that found in the $dv_{1g}$ and $dv_{1h}$ vesicles. The $dv_{am}$ and $dv_{amn}$ vesicles stain more intensely, suggesting that if these latter vesicles contain wall material it is modified in some way prior to actual incorporation. B. Golgi material at the lobe base from a section stained with phosphotungstic acid. Dark vesicles ($dv$) and large vesicles ($lv$) are associated with the Golgi cisternae. A, $\times 22100$; B, $\times 35300$; bars, 0.5 $\mu$m.
Table 1. Vesicle types in developing lobes

<table>
<thead>
<tr>
<th>Name</th>
<th>Contents</th>
<th>Size (µm)</th>
<th>Relative abundance (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$dv_{am}$, dark vesicle</td>
<td>Electron-dense interior, electron-lucent periphery</td>
<td>Oval, 0.15-0.35</td>
<td>4</td>
</tr>
<tr>
<td>$dv_{ak}$, dark vesicle</td>
<td>Electron-dense with electron-lucent inclusion</td>
<td>Oval, 0.15-0.35</td>
<td>5</td>
</tr>
<tr>
<td>$dv_{ag}$, dark vesicle</td>
<td>Moderately electron-dense, fibrous</td>
<td>Oblong, 0.3-0.45; circular cross-section, 0.15-0.2</td>
<td>5</td>
</tr>
<tr>
<td>$dv_{ak}$†, dark vesicle</td>
<td>Electron-dense, amorphous</td>
<td>Circular, 0.15-0.35</td>
<td>36</td>
</tr>
<tr>
<td>$pv$, pore vesicle</td>
<td>Moderately electron-dense, homogeneous</td>
<td>Circular, 0.2-0.4</td>
<td>2</td>
</tr>
<tr>
<td>$cv$, coated vesicle</td>
<td>Electron-lucent with coated membrane</td>
<td>Circular, 0.05-0.15</td>
<td>36</td>
</tr>
<tr>
<td>$lv$, large vesicle</td>
<td>Electron-lucent, faintly fibrillar</td>
<td>Oval, 0.35-0.5; when swollen, 0.7-1.2</td>
<td>10</td>
</tr>
</tbody>
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* Percentage of total number of vesicles counted (12,987) within the first 8 µm from the tips of lobes from cells at either the 5- or 9-lobe stage.
† $dv_{ak}$, previously unnamed.

Variance (Snedecor & Cochran, 1980) of the data showed that the spatial distribution of each vesicle type was non-uniform. Significance levels were $P < 0.01$ for the coated vesicles ($cv$), and $P < 0.0001$ for all other vesicle types. Furthermore, $t$-tests using Bonferroni probabilities (Sachs, 1982) for pairwise comparison of means indicated that: (1) the increased concentration of dark vesicles ($dv_{am}$, $dv_{ak}$, $dv_{ag}$, $dv_{ak}$) at the lobe tips was significant ($P < 0.001$); and (2) the increased concentration of large vesicles ($lv$) and pore vesicles ($pv$) at the lobe base was significant ($P < 0.001$). These points demonstrate quantitatively that in expanding *Micrasterias* lobes the distribution of vesicle types is highly ordered. Although we, and also Kiermeyer (1970a, 1981) and Menge (1976), have distinguished several categories of dark vesicles, the distribution patterns of each dark vesicle class were not significantly different from any other. Some or possibly all dark vesicle classes may be minor variations of a vesicle category with similar function.

Fig. 6. Quantification of vesicle distribution from 13 PTA-stained sections of lobes from cells at the 5- or 9-lobe stage. The peaks in dark vesicle ($dv$) concentration at the lobe tip (A), as well as the increase in pore vesicle ($pv$) and the large vesicle ($lv$) concentration towards the lobe base (B) are significant. See the text for statistical analysis. A. (□) $dv_{am}$; (○) $dv_{ak}$; (△) $dv_{ag}$; (+) $dv_{ak}$. B. (◇) $pv$; (▽) $cv$; (■) $lv$. 
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A

Normalized vesicle concentration

Distance from tip (μm)

B

Normalized vesicle concentration

Distance from tip (μm)
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Organization in the cytoplasm was not confined to the distribution of vesicles; lobes from developing semicells at the 5- to 33-lobe stage also consistently possessed an ordered arrangement of organelles (Fig. 7). Just below the tip region rough endoplasmic reticulum appeared. Typically, mitochondria were more centrally concentrated, followed basally by Golgi material and then the chloroplast. Measurements of the distribution of mitochondria and Golgi material were made in cells at the 5- or 9-lobe stage to confirm these qualitative observations (Fig. 8).

Effects of A23187 on cell ultrastructure

Electron-dense vesicles were completely dispersed throughout the lobe cytoplasm when cells were exposed to A23187 in 2 mM-CaCl₂ (Fig. 9A). In contrast, as observed in untreated cells (Fig. 3), a single cluster of these vesicles remained at the tip.

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Fig. 7. Section from a cell at the 5-lobe stage. Rough endoplasmic reticulum (rer) appears behind the electron-dense vesicle-rich tip zone. Further back, mitochondria (m) and then Golgi material (G) are present. ×13300; bar, 1 μm.

Fig. 8. Quantification of the distribution of mitochondria and Golgi material in 13 PTA-stained sections of lobes from cells at either the 5- or 9-lobe stage. Histogram bars represent the fraction of the total concentration of mitochondria (filled bars) or Golgi material (hatched) at that distance from the lobe tip.
Fig. 9. Patterns of electron-dense vesicles in A23187-treated cells at the 5-lobe stage. A. Cell fixed after 10 min in A23187 + 2 mM-CaCl₂. The electron-dense vesicles are dispersed throughout the lobe cytoplasm. B. Cell fixed after 10 min in A23187 + 2 mM-MgCl₂. Some dispersion of electron-dense vesicles occurs, but the tip aggregation of these vesicles is still apparent (open arrowhead). Compare with untreated cells (Fig. 3). A,B, x3690; bars, 3 μm.

of each lobe from cells treated with A23187 in 2 mM-MgCl₂ (Fig. 9B). These effects were quantified by determining electron-dense vesicle concentrations as a function of distance from the lobe tip (Fig. 10). An analysis of variance confirmed that the spatial distribution of vesicles was non-uniform in lobes from cells treated with A23187 in 2 mM-MgCl₂ (P < 0.0001), but did not differ significantly from uniformity in cells treated with A23187 in 2 mM-CaCl₂.

A23187 exposure also disturbed the normal cytoplasmic distribution of mitochondria and Golgi material. Similar effects occurred in 2 mM-CaCl₂ or 2 mM-MgCl₂. Mitochondria and Golgi material were found closer than normal to the lobe tips although, as in untreated cells, Golgi material was generally located proximally to mitochondria.

Cells exposed to A23187 also showed abnormal deposition of wall material along the cell wall margin (Fig. 11A). Cells treated with A23187 in 2 mM-CaCl₂ deposited wall material around the entire lobe periphery, in correspondence with the dispersed distribution of electron-dense vesicles in these cells (Fig. 11B). In cells treated with A23187 in 2 mM-MgCl₂, wall deposition was largely restricted to the tip, again in correspondence with the distribution of electron-dense vesicles in these same cells (Fig. 11C).
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Fig. 10. Average concentration of electron-dense vesicles as a function of distance from the lobe tip (7 lobes quantified per treatment). (○) Control; (□) A23187 + 2 mM-MgCl₂; (△) A23187 + CaCl₂. Electron-dense vesicles are concentrated at the lobe tip in control cells, and in cells treated with A23187 + 2 mM-MgCl₂. These vesicles are dispersed randomly throughout the cytoplasm in lobes from cells treated with A23187 + 2 mM-CaCl₂. See the text for statistical analysis.

Finally, cells exposed to A23187 in either 2 mM-CaCl₂ or 2 mM-MgCl₂, and then washed, partially recovered, with individual lobes branching up to three times more. Some cells produced lobes that jutted out from the normal plane of the semicell (Fig. 12).

DISCUSSION

The results of the present study demonstrate that developing Micrasterias cells, when adequately prepared for electron microscopy, possessed an ordered cytoplasmic organization during most of morphogenesis. We observed a single cluster of electron-dense vesicles at the tip of each expanding lobe, where wall deposition is normally localized. In lobes at later stages, which were about to branch, we observed a bilateral distribution of electron-dense vesicles prior to any detectable dichotomy in the cell wall. These electron-dense vesicles are thought to contain cell wall material (Kiermayer, 1970a,b; Menge, 1976). In agreement with this possibility, we found that phosphotungstic acid stained these vesicles, the cell wall and the plasma membrane. We also observed that, in the presence of the ionophore A23187, the cytoplasmic pattern of electron-dense vesicles (either tip-associated or dispersed) was well correlated with the sites of wall deposition (Fig. 11). Together, these results are consistent with the hypothesis that the electron-dense vesicles are involved in the
formation of the cell's primary wall. Since wall expansion probably requires deposition of new wall material as well as loosening of the old wall (Fève, 1979), the electron-dense vesicles may contain materials for both of these processes.

In addition to the electron-dense vesicles, the pore vesicles (pv) and large vesicles (lv) were also partitioned in the lobe cytoplasm. The increased basal concentration of these vesicles is likewise consistent with their proposed role in pore formation (Dobberstein, 1973) and slime secretion (Kiermayer, 1970a), processes that occur only after lobe elongation has ceased.

Although our results have demonstrated the existence of an ordered cytoplasmic organization during most of morphogenesis in *Micrasterias*, the underlying mechanism of pattern formation remains a mystery. We did not observe clusters of electron-dense vesicles at the early bulge stage, yet cells at this stage produce a well-defined wall deposition pattern (Kiermayer, 1964). Evidently, vesicle clusters are not essential for patterning. The appearance of such clusters in lobes at all later stages of morphogenesis is probably a secondary indication of the normal growth process in these lobes.

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**Fig. 12.** Partial recovery of a cell originally at the 5-lobe stage treated with A23187 in 2 mM-CaCl₂. After a 10 min incubation the cell was washed through four solutions of (in this case) 2 mM-CaCl₂ and then left to develop in 2 mM-CaCl₂. Most lobes are bulbous but branch once or twice more after treatment. Occasionally, aplanar lobes develop (arrow). ×540; bar, 20 μm.

**Fig. 11.** A23187-induced deposition of wall material. A. Cell fixed after 35 min of A23187 treatment. High-magnification view of fibrous and globular components of wall material (wwm), which accumulate along the original primary wall (pw). B. Cell fixed after 35 min in A23187 + 2 mM-CaCl₂. Ionophore-induced deposition of fibrous wall material (arrowheads) occurs abnormally along the sides of this lobe. Correspondingly, electron-dense vesicles are scattered along the lobe sides and throughout the cytoplasm. C. Cell fixed after 35 min in A23187 + 2 mM-MgCl₂. Electron-dense vesicles are present at the lobe tip, as is the ionophore-induced deposition of fibrous wall material (arrowheads). Electron-dense vesicles are sparse along the upper side of the lobe, in correlation with a lack of wall deposition there. A, ×38700; bar, 0.5 μm; B,C, ×78300; bars, 1 μm.
At the five-lobe stage and beyond, we also observed a reduced number of dark vesicles at the presumptive cleft sites, consistent with the reduced growth rate there (Harrison & Lacalli, 1978). This conforms with the observation that wall material is normally deposited primarily at the tips of lobes, and not at the sites of notch formation (Kiermayer, 1964; Lacalli, 1975a). Yet during cleft formation the wall thickens and changes its curvature. These observations imply that other factors apart from patterned wall deposition must be involved in generating the mature Micrasterias shape.

Our results do provide some insight into one component of the patterning mechanism in Micrasterias. The observed zonation of vesicles and organelles in individual Micrasterias lobes is strikingly similar to the ultrastructural organization observed in a variety of tip growing cells (Sievers & Schnepf, 1981). Others have suggested that Micrasterias lobes elongate by tip growth (Lacalli & Acton, 1974; Lacalli, 1975a; Meindl, 1982); our results support that hypothesis. Our observation of two separate vesicle clusters in a single lobe just prior to branching (Fig. 4) implies that during branching a single site for tip growth splits into two separate sites (Lacalli, 1975a,b). Thus, our understanding of morphogenesis in Micrasterias would be enhanced if we knew, in molecular terms, what a site for tip growth is, and why these sites bifurcate.

It seems likely that the mechanism of growth localization would be similar in most tip growing cells. As observed with chlorotetracycline fluorescence, many such cells (Reiss & Herth, 1978, 1979b), including Micrasterias (Meindl, 1982), have locally high concentrations of membrane-associated Ca\(^{2+}\) at their tips. In a previous study (McNally et al. 1983), we found that in the presence of the ionophore A23187 wall expansion stops in Micrasterias, but wall deposition and cytoplasmic streaming continue. A23187 + Ca\(^{2+}\) cause wall material to accumulate rather uniformly around the cell periphery, while A23187 + Mg\(^{2+}\) do not significantly affect the normal pattern of tip-associated wall deposition.

In the present study, consistent with these earlier observations, we found that the distribution of electron-dense vesicles was dispersed by A23187 + Ca\(^{2+}\) but not A23187 + Mg\(^{2+}\). Reiss & Herth (1979a) have made similar observations in lily pollen tubes. Such a disruption of vesicle distribution by high calcium does not imply that calcium normally establishes this organization, since high intracellular calcium may non-specifically disrupt the normal patterning apparatus. However, all other non-specific effects of the ionophore can be ruled out by our data, since A23187 in the presence of magnesium did not substantially alter the electron-dense vesicle distribution, or the pattern of wall deposition (McNally et al. 1983).

Some evidence suggests that A23187 was acting specifically in our experiments. Ionophore treatment was not toxic, since cells that were washed partially recovered. Although A23187 induced some degradation of the cytoplasm in treated cells, this disruption occurred in the presence of calcium or magnesium, and so it could not be responsible for the altered vesicle distributions observed only in the presence of calcium. Ultimately, it is difficult to distinguish between specific and non-specific effects of any drug treatment. Our results are consistent with the hypothesis that
Cytoplasmic organization in *Micrasterias* is involved in organizing the distribution of electron-dense vesicles in *Micrasterias*, but more careful experiments are required to substantiate this theory.

**REFERENCES**


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