INVERSION IN VOLVOX TERTIUS:
THE EFFECTS OF CON A

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SUMMARY

During the development of Volvox tertius spheroids, a single-celled gonidium enlarges and undergoes multiple incomplete cleavages to give an embryo which is 'inside-out' with respect to the adult organism. A morphogenetic movement, termed 'inversion', turns this hollow ball of cells 'inside-out' through a hole, the phialopore. In V. tertius this phialopore possesses 4 inwardly directed lips.

Normal inversion was studied in vitro in slide chambers and involved cell-shape changes accompanied by the production of pseudopodia and the bending backwards of the phialopore lips.

100 µg/ml Con A specifically and reversibly blocked inversion. Despite the inhibitory effect on cell division, the blocking of inversion was not due to the blocking of the last cell division some 50–100 min prior to inversion. Neither did the first cell-shape change from pear- to spindle-shape appear blocked. A feature of inhibition by Con A was the enhanced production of pseudopodia by embryos blocked at inversion, and the abnormal production of pseudopodia by embryos blocked at earlier stages. Non-inverting embryos showed internal flagella. We suggest that the Con A block to inversion, which may be reversed by α-methyl mannoside, arises from the prevention of backwards-bending of the phialopore lips.

Fluorescein-isothiocyanate-Con A bound to embryo and cell coat, and more strongly to the embryo at pre-inversion. SDS-polyacrylamide gel analysis of proteins isolated from embryos showed 4 glycoprotein bands, but Con A binding to these bands could not be demonstrated.

INTRODUCTION

The mechanisms and control processes underlying morphogenesis are still not understood. Holtfreter (1943, 1944) suggested that co-ordinated cell-shape changes were responsible, whilst individual cell-shape changes have been attributed to cytoskeletal elements (Wessells et al. 1971). We have studied morphogenesis in Volvox because of the potential 'simplicity' of this event. While not implying homology as others have done (Haeckel, 1874), we consider Volvox may prove to be a valuable model system in the study of gastrulation and other morphogenetic movements in higher eukaryotes.

Volvox is a multicellular, photosynthetic autotroph consisting of only 2 cell types, biflagellate somatic cells arranged on the periphery of a spheroid and gonidia, asexual reproductive cells inside. The latter may give rise to reproductive cells on the appropriate stimulus. In asexual V. tertius spheroids, each gonidium divides to produce an embryo consisting of a hollow ball of cells which is really a syncytium since cyto-
kinesis is incomplete. This hollow ball is inside-out with respect to the adult conformation, with gonidia on the exterior and the future flagellate ends of the somatic cells directed inwards. A morphogenetic movement termed 'inversion' turns this hollow ball of cells inside-out through a hole, the phialopore.

Inversion in different Volvox species was described many years ago by Janet (1912, 1922, 1923) and Pocock (1933, 1938), while Kelland (1964) made the first experimental study through various micromanipulations of the embryo, and also made detailed observations of cell-shape changes in different regions of inverting embryos of V. aureus and V. globator. Recently, an impressive study of V. carteri by Viamontes & Kirk (1977) used scanning electron microscopy to study inversion. We thought that further insight into the nature of cell-shape changes during inversion might be gained by the use of a lectin, Con A.

Lectins have been used extensively in recent years to study cell surfaces because of their ability to bind to specific saccharide groups. Surprisingly few attempts have been made to assess the effect of lectins on plant cell surfaces despite their almost exclusive occurrence in plant tissues. A notable exception is the study of gamete agglutination reactions in Chlamydomonas by Wiese & Wiese (1975). The literature contains numerous examples of the inhibitory action of lectins as well as the better known example of mitogenic stimulation. Amongst the inhibitory actions known are inhibition of growth (as measured by DNA and RNA synthesis) in certain cancer cell lines (Ralph & Nakoinz, 1973), inhibition of mitogenic stimulation of lymphocytes at high concentrations of Con A, and inhibition of the morphogenetic movement in 'eversion' in Drosophila imaginal discs (Mandaron, 1974).

MATERIALS AND METHODS

V. tertius was obtained from the Collection of Algae and Protozoa, Cambridge, England. Axenic clones were isolated (Starr, 1964) and cultures grown in sterile volvox medium (s.v.M.) (Darden, 1966). Five-millilitre cultures in test tubes were maintained at 25 °C and provided with continuous incident illumination of 2000 lux. They were subcultured weekly to ensure continued asexual development, since this species is homothallic.

Embryos at various stages were obtained through rupture of the parent by drawing the spheroid up a narrow-bore glass pipette. Subsequently the embryos were transferred to small paraffin chambers for observation with a ×40 phase-contrast objective (Ireland & Hawkins, in press). For each experiment fresh stock solution of Con A (100 μg/ml) was prepared in s.v.m. and diluted as required. To monitor the effect of Con A, batches of different Volvox stages were set up in paraffin chambers and later scored blind for the stage of development. Embryo cell density was estimated by counting the average number of cells in a square of a grid graticule fitted to the microscope eyepiece. Embryo cell number was calculated in the following way. An average measure of the embryo circumference was obtained from opistometer measurements of camera lucida drawings of 3 orthogonal faces of the embryo. The surface area was calculated then from the average circumference treating the embryo as a sphere and ignoring the phialopore. The embryo cell number was calculated from the embryo surface area and the cell density of the surface.

To prepare sufficient proteins for electrophoresis, quantities of young embryos were extracted from Volvox grown in bulk cultures (Ireland, 1978). The embryos were collected by centrifugation, resuspended in 0.125 M Tris-HCl buffer, pH 6.8 and lyed by the addition of sodium dodecyl sulphate (SDS) to a final concentration of 2.5 %. The lysate was shaken for 5 min and then immersed in a boiling water bath for 2 min to form SDS/protein complexes. It was spun to remove insoluble material and later placed in boiling water in sample buffer
Con A and inversion in Volvox (Laemmli & Favre, 1973) containing 2-mercaptoethanol. The sample was loaded onto 8% polyacrylamide rod gels provided with 2.5% stacking gels. The gels were subjected to electrophoresis (2 mA/gel) in a discontinuous buffer system modified from Laemmli & Favre (1973). Replicate gels were stained either with Coomassie brilliant blue (0.15% in 45% methanol, 9% acetic acid) or a modified PAS technique (Kapitany & Zebrowski, 1973) to detect glycoproteins. Molecular weights of Volvox proteins were calculated on the basis of known protein standards (Dunker & Rueckert, 1969; Weber & Osborn, 1969).

In order to examine the binding of Con A to various stages of developing Volvox, fluorescein isothiocyanate-Con A (FITC-Con A, Miles-Yeda Ltd.) was employed. Isolated embryos were treated with FITC-Con A for varying lengths of time, washed in S.V.M. and observed with a Zeiss microscope illuminated by an ultraviolet source with BG III filters inserted.

RESULTS

Inversion in Volvox tertius

Inversion in Volvox tertius was investigated in embryos removed from their parent spheroids and prepared for observation in paraffin chambers, and in intact Volvox. The embryo before inversion was variable in shape, but usually roughly spheroidal although slightly compressed in the anterior–posterior axis. At the future posterior pole of the embryo the phialopore was clearly visible and bound by 4 inwardly directed lips. The surface of the embryo often possessed several shallow concavities (Fig. 1 A) and 1 or 2 isolated cells possessed a long cytoplasmic process or pseudopodium. Imminent inversion was made apparent by 3 probably related events, a slight overall shrinkage, a more rounded appearance of the embryo (Fig. 1 B) and the increased clarity of the cell borders. The latter was the first indication of a cell-shape change; the cells became more spindle shaped radially through the production of a short pseudopodium and more rounded in surface view. Although pseudopodia appeared over the entire outer surface, they were longer and more easily seen in the future anterior half of the embryo. The production of colourless pseudopodia gave the embryos a 'spikey' appearance characteristic of early inversion, Fig. 1 C. During this time the phialopore had enlarged and later a constriction appeared between the phialopore region and the equatorial region of the embryo, Fig. 1 D. The phialopore widened further and the lips of the embryo bent back until a characteristic 'hat' stage was reached, Fig. 1 E, F. The pseudopodia in the future anterior region (Fig. 1 G) elongated, while those in the future posterior region disappeared. The embryo continued to turn inside-out through a 'torus' stage (Fig. 1 H, I) until a small spherical embryo resulted, Fig. 1 J.

During inversion each cell became flagellated, observable first on the lips. Subsequently, the length of the flagella increased (Fig. 1 K) and the production of cell wall matrix pushed the cells apart and expanded the young spheroid (Fig. 1 L).

Effect of Con A

‘Pre-inversion’ embryos were selected using the criteria of embryo and cell size, and embryo and phialopore shape. When these embryos were prepared and maintained in slide chambers, a high percentage, 80–100, depending on the batch, always inverted within 4 h. When control and Con A-treated batches of embryos were compared, it was found that concentrations of 20 μg/ml Con A and above prevented
inversion, whereas with a concentration of 10 \mu g/ml, the inversion process was not significantly different from that of control embryos. In addition, observations of embryos treated with concentrations of Con A above 20 \mu g/ml showed a preponderance with medium to long pseudopodia. These pseudopodia were observed in the electron microscope (Ireland, 1978) and appeared similar in structure to the pseudopodia of normal inverting embryos containing part of the chloroplast drawn-out into the pseudopodium.

Fig. 1. Inversion in *Volvox tertius*. A–C, E, F, H–J, L are of one embryo, times given in parentheses, and D, G, K are of another embryo. All \times 530. (A) Pre-inversion embryo viewed from the future anterior pole. The irregularly shaped embryo is contained within a vesicle (arrow). (12.45). (B) Early inversion stage. The embryo is now more rounded and the phialopore has widened. Final cell division has taken place. (13.35). (C) Side view of (B): the short colourless pseudopodia (arrows) in the future anterior half give the embryo a ‘spiky’ appearance (13.35). (D) A later inversion stage: the constriction behind the phialopore is clearly seen. (E) A ‘hat’ stage viewed into the phialopore showing that the lips (arrows) have bent back (13.45). (F) Side view of E. At the top, one lip is bent back on itself. Pseudopodia are still present (arrows) (13.45). (G) A later ‘hat’ stage showing long pseudopodia (arrows). (H) A ‘torus’ stage (side view) (14.05). (I) Phialopore view of (H) (14.05). (J) Inversion nearly complete: the phialopore is not completely closed. Short flagella are present on the outer surface (14.50). (K) Post-inversion spheroid: flagella are longer and cells have begun to move apart due to secretion of matrix material. (L) Post-inversion spheroid: individual somatic and larger gonidial cells (arrow) are now visible (10.00, next day).
These initial observations raised several points which required further investigation:
(a) Since the timing of the last cell division before inversion and the effect of Con A on division were unknown, inhibition of inversion could be due to the inhibition of cell division. (b) It was not clear whether the inhibition was due to a toxic effect of Con A or whether inhibition was reversible. (c) Although the embryo had been removed from the parental spheroid it still retained an embryo coat (or vesicle) and thus it was uncertain to which surface the Con A was binding. (d) The preponderance of pseudopodia seen in Con A-treated embryos might be due to blocked inversion or to the increased induction of pseudopodia.

![Diagram](image.png)

Fig. 3. The timing of pseudopodia production in Con A-treated and control embryos, and the inhibition of inversion. A. h in 100 μg/ml Con A; B. h in control medium; a. % embryos with pseudopodia; b. cumulative % embryos that have shown many pseudopodia; c. % embryos at inversion and post-inversion. a. a few cells with pseudopodia; b. most cells with pseudopodia; c. % inversion and post-inversion.

To investigate the timing of the last cell division before inversion, cell density was measured in normal pre-inversion embryos and was found to increase significantly before inversion. Surface area was measured also, and when embryo cell numbers were calculated from this data, there was evidence for a final cell division 50–100 min before inversion (Fig. 2). There was a slight decrease in surface area before inversion but this was insufficient to explain the increase in cell density. The error due to ignoring the phialopore was estimated to be approximately 2%. It was apparent that inverting embryos possessed variable numbers of cells, as did adults. In post-inversion spheroids the estimated range was between 338 and 613. It was clear that a batch of pre-inversion embryos would have included both embryos that had completed their final cell division and those that had not.

We looked first at the effect of Con A on cytokinesis in very early stages: in small gonidia (diameter 23.5 μm), development was slowed by 10 μg/ml and completely inhibited by 100 μg/ml even though enlargement was unaffected. Large gonidia (diameter 32.9 μm) just prior to division were unaffected by 25 μg/ml, but were
Con A and inversion in Volvox

completely inhibited by 50 \( \mu g/ml \). When Con A was applied to later embryos, 8–128 cells, development was slowed by 20 \( \mu g/ml \) and completely inhibited by 30 \( \mu g/ml \). In young embryos (8–128 cells) treated with Con A pseudopodia were often seen, although never seen in control embryos of the same age. This suggested enhanced production of pseudopodia in Con A-treated embryos. When pre-inversion embryos were treated with 100 \( \mu g/ml \) Con A, the proportion of embryos showing pseudopodia was similar to that seen in batches of control embryos, which by this time were inverting (Fig. 3). So, with pre-inversion stages, although some enhanced early pseudopodial production was seen, most pseudopodial production was correlated with the expected inversion.

Table 1. The effect of different concentrations of Con A on V. tertius embryos of known cell density

<table>
<thead>
<tr>
<th>Concentration of Con A, ( \mu g/ml )</th>
<th>Stage at start</th>
<th>Prel (3–4)*</th>
<th>Prel (6)</th>
<th>Early I</th>
<th>Late I</th>
<th>Post-Inv.</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Prel (3–4)*</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Prel (6)</td>
<td>--</td>
<td>2</td>
<td>--</td>
<td>--</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Early I</td>
<td>--</td>
<td>1</td>
<td>1</td>
<td></td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Late I</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>30</td>
<td>Prel (3–4)*</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Prel (6)</td>
<td>--</td>
<td>4</td>
<td>1</td>
<td>--</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Early I</td>
<td>1</td>
<td>--</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Late I</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>Prel (3–4)*</td>
<td>14</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Prel (6)</td>
<td>1</td>
<td>1</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Early I</td>
<td>--</td>
<td>1</td>
<td>2</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Late I</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1</td>
<td>--</td>
</tr>
</tbody>
</table>

* Number in parentheses indicates cell density/91 \( \mu m^2 \). Prel = Pre-inversion; Early I = Early inversion stage; Late I = Late inversion stage.

Individual embryos from pre-inversion batches were known to invert at variable times since cultures were not synchronous and staging was not accurate enough to select a more homogeneous batch. Since the final cell division was now known to occur shortly before inversion, and since division was inhibited by Con A, it became important to try and identify more exact stages. In an attempt to do this, the effect of Con A was monitored on embryos of known cell density, Table 1. Now higher concentrations of Con A were required to reduce inversion significantly. Some embryos were able to invert in 30 \( \mu g/ml \), while all inversion was inhibited by 100 \( \mu g/ml \).

The specificity and reversibility of Con A inhibition was tested by use of the hapten, \( \alpha \)-methyl mannoside. This saccharide is strongly bound by Con A thus inhibiting other Con A-binding reactions. The saccharide was mixed with Con A in the molar ratio of 55000:1 and a comparison made with the effects of untreated Con A. The inhibitory action of Con A was considerably reduced in the presence of \( \alpha \)-methyl mannoside (Table 2). However this level of hapten also had some effect on development, in particular it inhibited the expansion of the spheroid (Table 2).
Embryos could also be released from the Con A-induced inhibition of inversion by treatment with α-methyl mannoside (Table 3). When batches of late multicellular embryos, pretreated with 20 μg/ml Con A, were placed in α-methyl mannoside the responses were of 2 kinds. Embryos either started inversion in 1–5 min, and which was often abnormal, or inversion took place more than 60 min later. Abnormal inversion occurred when the early events took place more quickly and violently often leading to 'splits' in the embryo and to 2 or more embryos in several cases.

Table 2. *Con A inhibition of inversion in V. tertius; competition with α-methyl mannoside*

<table>
<thead>
<tr>
<th>Medium*</th>
<th>Pre-Inv.</th>
<th>Inversion</th>
<th>Post-Inv.</th>
<th>Normal expansion of spheroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con A (100 μg/ml)</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Con A (100 μg/ml) + α-mm (19.4 mg/ml)</td>
<td>1</td>
<td>1</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>α-mm (19.4 mg/ml)</td>
<td>1</td>
<td>0</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Control (S.V.M.)</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

*α-mm = α-methyl mannoside; s.v.m. = sterile volvox medium.
†Stage reached 17.5 h after pre-inversion embryos set up in various media.

Table 3. *Effect of α-methyl mannoside after Con A inhibition of inversion in V. tertius*

<table>
<thead>
<tr>
<th>h in Con A, 20 μg/ml</th>
<th>Normal medium</th>
<th>No. in exp.</th>
<th>Medium + α-mm</th>
<th>No. in exp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>9</td>
<td>8 (a)</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>9</td>
<td>7 (b)</td>
<td>13</td>
</tr>
</tbody>
</table>

*Number reaching post-inversion 17 h after initial treatment with Con A. Subsequent development in normal medium or medium + α-methyl mannoside. (a) 25% of these embryos inverted immediately after removal from Con A. (b) 54% of these embryos (i.e. all that did eventually invert) inverted immediately after removal from Con A.

Embryos which had been inhibited from inversion by Con A for several hours often had a characteristic appearance which we termed the 'strained state'. Although few pseudopodia were present, the cells were very elongate and these embryos inverted quickly when released from the Con A block. They did not form pseudopodia during inversion. Embryos treated with near threshold levels of Con A often became flagellated over their entire inner surface despite failure to invert. Clearly flagella formation was not a prerequisite for inversion.

Fluorescein-labelled Con A was used to visualize the regions of the embryo that bound the lectin. In gonidia the fluorescence was distributed evenly over the surface and seemed to bind more to the cell membrane than the cell coat, although this was difficult to see. In later multicellular stages, the cell coat seemed to bind Con A more strongly. In pre-inversion stages fluorescence was located on the coat and more
strongly on the embryo, seen as an intercellular lattice in surface view. Pseudopodia did not seem to bind Con A but this was difficult to visualize in the whole embryo due to light scatter.

**SDS-polyacrylamide gel electrophoresis**

Since all stages of development seemed to bind Con A to some extent we decided to try and isolate a possible receptor. A large quantity of mixed-stage embryos were obtained (Ireland, 1978) and proteins prepared for SDS-polyacrylamide gel electrophoresis. A comparison of gels stained for proteins and glycoproteins showed 4 high-molecular-weight bands that were probably glycoproteins. However an attempt to demonstrate Con A-binding of these or any other bands using the technique of West & McMahon (1977) was unsuccessful.

**DISCUSSION**

Although inversion occurs in many members of the Volvocales, details of the process differ even between *Volvox* sp. The most significant difference is in the initial position of the 'bend zone'. In *V. aureus* it is initiated at the phialopore lips, whereas in *V. globator* it starts on the equator (Kelland, 1964). Our observations on *V. tertius* agree closely with those of Pocock (1938) and suggest that the bend region starts behind the region of the phialopore which enlarges considerably before the lips bend back. In this respect *V. tertius* differs from *V. carteri* which follows the *V. aureus* pattern (Viamontes & Kirk, 1977). Furthermore the phialopore in *V. tertius* is not only prominent before inversion, but consists of inwardly directed flaps unlike that in *V. carteri*.

Kelland (1964) carefully documented the cell-shape changes from different regions of *V. aureus* and *V. globator* embryos, before, during and after inversion. From these results he suggested that cell-shape changes were the motive force behind inversion and that a co-ordinated wave of cell-shape changes was responsible for the observed process. The transmission and scanning electron-microscope observations of Viamontes & Kirk (1977) supported these ideas. In addition they drew closer attention to the cells in the bend region and their characteristic flask-shape, also seen in the gastrula of other embryos, which are not easily seen by direct observation.

Viamontes & Kirk (1977) suggested 4 phases of cell-shape change. First, an initial pear- to spindle-shape change causing contraction of the embryo and widening of the phialopore. Second, a transformation from spindle- to flask-shape starting at the phialopore lips with a simultaneous migration of the cytoplasmic connections from the mid-cell line to the pseudopodial stalk tips. This, they said, led to the cells fanning out at their flagellate ends and thus bending back the lips of the phialopore, whilst this region of flask-shaped cells, the bend region, moved distally away from the phialopore. Third, more proximal cells changed from flask-shaped to a more columnar form. In the fourth phase, cells changed from columnar to cuboidal which increased the embryo spheroid diameter.

We have shown that Con A specifically and reversibly inhibits inversion. For,
despite the inhibitory effect of Con A on division, the blocking of inversion was not due to the blocking of the last cell division, since this was shown to have finished between 50 and 100 min before inversion, while Con A can block inversion after this. Pickett-Heaps (1970) stated that it was difficult to estimate the time between the final cell division and the start of inversion, but our figure agrees reasonably well with the 30-60 min given by Viamontes & Kirk (1977) for _V. carteri_, although these authors do not state the method they used.

We have also shown that embryos very close to or undergoing inversion can be blocked. The cell-shape change from pear- to spindle-shaped was not blocked by Con A; in fact it may be enhanced since the most interesting feature of Con A-treated embryos was pseudopodium production. In older embryos this can be explained by the accumulation of embryos at the inversion stage as a result of the blocking action. However this cannot explain the observation of pseudopodia in younger embryos which seems probably to be due to enhanced production.

The cell-shape change from pear- to spindle-shape presumably involves microtubules. Pickett-Heaps (1970) observed microtubules in the long axis of cells from inverting male spheroids of _V. tertius_. They have also been observed in projections of spindle-shaped cells of _V. carteri_ pseudopodia (Viamontes & Kirk, 1977) and in _V. tertius_ (Ireland, 1978).

We think that Con A may inhibit inversion by preventing the phialopore lips from bending back. How this arises is not clear, although several possibilities can be suggested. First, since Con A is tetrameric at the pH used, it could merely 'glue' the cells together and prevent them moving relative to one another. Secondly, Con A could inhibit flask-cell formation by preventing the movement of cytoplasmic connections to the tips of the pseudopodia. Some support for this idea may come from observations of 'strained' embryos which were seen after prolonged treatment with Con A. In these embryos the cells were extremely elongated and, when released from the block to inversion by α-methyl mannoside, sometimes inverted violently breaking their cytoplasmic connections and forming 2 or more embryos. This might result if the connections had not been able to move quickly enough following the release from inhibition and were just pulled apart. Thirdly, Con A may be affecting the cortical cytoskeleton indirectly via a surface-modulating assembly (Edelman, 1976), perhaps by causing actin polymerization in the wrong place and thus altering the normal shape change. Actin has not been identified in *Volvox* sp although it has been found in other algae (Kersey, 1974; Palevitz, Ash & Hepler, 1974; Williamson, 1974).

We have isolated proteins from _V. tertius_ embryos and have demonstrated at least 4 glycoprotein bands. However we have not been able to demonstrate Con A-binding by any of these bands. Comparisons with the molecular weights of cell-wall glycoproteins isolated from *Chlamydomonas* (Catt, Hills & Roberts, 1976) suggest that these may be the cell-wall glycoproteins of _V. tertius_.

Normally the production of flagella is associated with the third cell-shape change, namely from flask-shaped to cuboidal. We have obtained non-inverting embryos which became internally flagellated showing that flagella production can occur without inversion. Griffin & Huskey (1974) have produced flagella-less mutants which invert
Con A and inversion in Volvox normally. These results taken together clearly show that the flagella have no role to play in inversion, as once suggested by Pocock (1933).

The production of pseudopodia by young embryos suggests that cells are capable of pseudopodial production at this stage. This may give us an insight into how the process of inversion is controlled. Perhaps a multivalent inducer protein is made which promotes pseudopodial production by acting externally, or Con A is only a mimic for internal changes involving the cytoskeleton at inversion.

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REFERENCES


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