Unequal cleavage in leech embryos: zygotic transcription is required for correct spindle orientation in a subset of early blastomeres

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SUMMARY

Leech embryos undergo invariant sequences of equal and unequal cell divisions to give rise to identifiable progeny cells. While many of the early cleavages are under maternal control, the divisions of a subset of early blastomeres (the large cells of the D′ lineage) are perturbed after the inhibition of zygotic transcription. Analysis of the different types of cells produced in embryos injected with the transcriptional inhibitor, α-amanitin, revealed that the symmetry of cell division is perturbed in these large D′-derived cells during this early period of development.

These cells, which would normally undergo a series of equal and unequal cleavages, always undergo equal cleavages after the inhibition of zygotic transcription. It appears that zygotically transcribed gene product(s) are required in the large cells of the D′ lineage to orient the mitotic spindles properly for these unequal cell cleavages.

Key words: asymmetric cell division, transcriptional inhibition, α-amanitin, mitotic spindle, spindle position, centrosome rotation, Helobdella triserialis

INTRODUCTION

The generation of cell diversity is fundamental to embryonic development. Sister cells can acquire different developmental fates extrinsically through interactions with neighboring cells or intrinsically by being different from the time they are produced. Cells with intrinsic differences can be generated by a variety of mechanisms, including the production of cells that differ in size as the result of unequal cleavage. Unequal cleavages are common during embryonic development; examples include the formation of polar bodies during oogenic meiosis and the formation of micromere/macromere pairs in some species of annelids, molluscs, echinoderms and ascidians. The two differently sized cells generally follow different developmental pathways. For example, the four micromeres produced at third cleavage in leech embryos give rise to the progenitors of the nonsegmental cephalic structures, whereas the macromeres give rise to the progenitors of the segmental body tissues (Weisblat et al., 1984; Nardelli-Haefliger and Shankland, 1993).

In addition to the unequal third cleavage, there are many other unequal cell divisions during leech development (Whitman, 1878; Weisblat et al., 1978; Fernandez, 1980; Sandig and Dohle, 1988; Bissen and Weisblat, 1989). Three of the macromeres (A′, B′ and C′) of the eight-cell embryo undergo two more highly unequal cleavages to generate the secondary and tertiary trios of micromeres (see Fig. 1). The fourth macromere (D′) undergoes a highly organized series of unequal and equal cleavages during stages 4–6 to generate fifteen additional micromeres and five pairs of embryonic stem cells or teloblasts. The teloblasts also divide unequally; each undergoes a repeated series of unequal cleavages to generate a long chain or bandlet of segmental founder cells called primary blast cells. Little is known about how these complex patterns of equal and unequal cell divisions are regulated.

Previous work has shown that the first sign of a requirement for zygotic transcription in leech appears during the cleavages that give rise to the teloblasts (Bissen and Weisblat, 1991). Stage 1 embryos injected with the transcriptional inhibitor, α-amanitin, appear to develop normally until about stage 5, at which time the divisions of the large cells of the D′ lineage become perturbed and supernumerary medium-sized cells are generated. These cells resemble teloblasts in that they contain both yolk-rich cytoplasm and yolk-deficient cytoplasm or teloplasm, rather than resembling micromeres or blast cells, which contain mainly teloplasm and little yolk-rich cytoplasm.

The supernumerary yolk-rich cells in the α-amanitin-injected embryos could have arisen as a result of increased rates of cell division. If so, the α-amanitin-injected embryos would contain extra yolk-filled cells, in addition to the normal complement of D′-derived cells. Extra cell divisions have been observed in Drosophila and Caenorhabditis elegans embryos that were exposed to α-amanitin prior to the onset of zygotic transcription (Edgar and Schubiger, 1986; Edgar et al., 1994).

Alternatively, the extra yolk-rich cells in the α-amanitin-injected leech embryos could have arisen because of alterations in the symmetry of cell division. If the large cells of the D′ lineage divided at the correct time but always divided equally, rather than undergoing their normal sequences of equal and unequal cell divisions, the embryos would contain the same...
number of cells as the control embryos. The experimental embryos, however, would contain more large yolk-rich cells and fewer small yolk-deficient cells.

To distinguish between these two possibilities, we determined the total number of large yolk-rich and small yolk-deficient cells in the D' lineage of stage 5 control and α-amanitin-injected embryos. Here we present data supporting the second hypothesis that inhibition of zygotic transcription alters the symmetry, but not the rate, of cell division in the large cells of the D' lineage during stage 5. In the absence of zygotic transcription, these cells always cleave equally to generate progeny cells of similar sizes. These cells undergo equal cleavages because their mitotic spindles do not become positioned with one pole closely apposed to the cell cortex as do those of the unequally dividing control cells. Zygotically transcribed gene product(s) appear to be required in the large D'-derived cells during stage 5 to orient the mitotic spindles properly for asymmetric cleavage. These unequal cleavages of the large cells of the D' lineage have an absolute requirement for zygotic transcription, whereas the earlier micromere-forming and the later blast cell-forming unequal cleavages do not.

MATERIALS AND METHODS

Embryos were obtained from a laboratory breeding colony of Helobdella triserialis and grown at 23°C in a buffered saline medium (Weisblat et al., 1980). The developmental staging system and cell lineage nomenclature used are those of Stent et al. (1992).

Zygotic transcription was inhibited by injecting stage 1 embryos with 75 μg/ml of α-amanitin (Sigma) as described earlier (Bissen and Weisblat, 1991). The progeny of macromere D', proteloblast DNOPQ or proteloblast DM were labeled with a lineage tracer by injecting the parental cell with either 75 mg/ml of tetramethylrhodamine-dextran-amine (RDA; Molecular Probes) or fluorescein-dextran-amine (FDA; Molecular Probes) and 100 mg/ml of fast green FCF (Sigma) in 0.2 M KCl.

The embryos were fixed in 4% formaldehyde and 50 mM sodium cacodylate, pH 7.4, at room temperature for 1 hour. They were then incubated (Nelson and Weisblat, 1991) with a monoclonal antibody (provided by D. Stuart, University of California, Berkeley) that recognizes leech cell nuclei. The monoclonal antibody was detected with either fluorescein-conjugated rabbit anti-mouse secondary antibodies (1:250 dilution; ICN) or rhodamine-conjugated goat anti-mouse secondary antibodies (1:500 dilution; Boehringer-Mannheim). The nuclei were also counterstained with Hoechst 33258 (Sigma). Some embryos were dehydrated through graded ethanol solutions, embedded in JB-4 resin (Polysciences, Inc.), sectioned at 1.4 μm with a glass knife microtome and mounted with Gurr Fluoromount (Gallard Schlesinger, Inc.). Other embryos were dehydrated through a series of ethanol solutions, cleared in a solution of benzyl benzoate and benzyl alcohol (3:2) and viewed as whole mounts.

Images were collected on a Zeiss Axioskop microscope with a cooled CCD camera (Photometrics) using IPLab Spectrum acquisition software (Signal Analytics Corp.) and were digitally deconvolved using Micro-Tome software (VayTek, Inc.).

RESULTS

Inhibition of transcription converts unequal cleavages into equal cleavages in stage 5 embryos

At the beginning of stage 5 a normal embryo contains four D'-derived large blastomeres (two NOPQ proteloblasts and two M teloblasts); five D'-derived micromeres; two D'-derived (m)
blast cells; three macromeres (A″, B‴ and C‴); and ten other micromeres (the primary quartet, secondary and tertiary trios) (see Fig. 1). During stage 5 each NOPQ proteloblast undergoes two unequal cleavages to generate two micromeres and then cleaves nearly equally into an N teloblast and an OPQ proteloblast (stage 6a). Each M teloblast also undergoes three unequal cleavages during stage 5 to produce three additional m blast cells. Some of the primary quartet micromeres also divide during stage 5 (Sandig and Dohle, 1988).

To determine whether the supernumerary yolk-rich D‴-derived cells present in α-amanitin-treated embryos had arisen as a result of increased rates of cell division or altered planes of division, the total number and types of cells were determined in stage 5 control and experimental embryos. Transcription was blocked by injecting stage 1 embryos with α-amanitin and the D‴-derived cells were labeled by injecting the parental cell (D‴) with a fluorescent lineage tracer at stage 4a. Both groups of embryos were fixed when the control embryos reached early stage 5 (14 hours after egg deposition; AED) or mid stage 5 (16 hours AED).

The total number of cells was determined in each embryo, and the cells were classified on the basis of lineage tracer-labeling, size, and cytoplasmic composition. The large lineage tracer-labeled cells that contained both teloplasm and yolky cytoplasm were classified as D‴-derived yolk-rich cells (Fig. 2A,B). The small tracer-labeled cells located at the animal pole were classified as D‴-derived micromeres (arrows in Fig. 2A). The small tracer-labeled cells located deep within the embryo were classified as D‴-derived (m) blast cells. The three very large, unlabeled cells were classified as the A″, B‴ and C‴ macromeres. The small unlabeled cells were classified as the primary quartet, secondary and tertiary trios of micromeres. Since the boundaries between the small yolk-free cells were difficult to discern, the number of cells was determined by counting the nuclei. The size and location of the nuclei were carefully compared in adjacent sections to ensure that each nucleus was counted only once.

The numbers of the different types of cells in the control and α-amanitin-injected embryos are presented in Table 1. The number of cells in the control embryos was identical to previously published figures for glossiphoniid leeches (Sandig and Dohle, 1988). As shown previously (Bissen and Weisblat, 1991), the α-amanitin-injected embryos contained more D‴-derived yolk-rich cells. Most (13/17) of the α-amanitin-injected embryos contained one or two extra D‴-derived yolk-rich cells at 14 hours AED. All of the experimental embryos contained extra D‴-derived yolk-rich cells at 16 hours; the number of extra yolk-rich cells ranged from five to 14, with an average of eight (see Fig. 2B). The α-amanitin-injected embryos also contained fewer small, yolk-deficient, D‴-derived cells. These experimental embryos contained all five of the expected D‴-derived micromeres at 14 hours, but no additional D‴-derived micromeres were produced by 16 hours, whereas there were two additional D‴-derived (nopq′) micromeres in the control embryos. The experimental embryos also contained few, if any, D‴-derived blast cells, in contrast to the four or six m blast cells in the control embryos at 16 hours.

Thus, the average number of D‴-derived cells was similar in
the control and experimental embryos at 14 hours (11 vs. 10, respectively) and at 16 hours (16 vs. 17, respectively), but the α-amanitin-injected embryos contained more large, yolk-rich, D*-derived cells and fewer small, yolk-deficient, D’-derived cells. At 16 hours, for example, the experimental embryos contained 12 large and five small D’-derived cells, whereas the control embryos contained four large and 12 small D’-derived cells. These data indicate that, after the formation of the NOPQ cells in the α-amanitin-injected embryos, the yolk-rich cells of the D’ lineage failed to divide unequally to produce small yolk-deficient and large yolk-rich cells, but rather they divided equally to produce similarly sized yolk-containing cells. It appears, therefore, that zygotically transcribed gene product(s) are required to direct the symmetry and not the rate of cleavage of the large D’-derived blastomeres during stage 5.

**Not all unequal cleavages require zygotic transcription**

Previous work suggested that embryos treated with α-amanitin at stage 1 cleaved normally until stage 5 (Bissen and Weisblat, 1991). To determine whether all of the unequal cell divisions that normally occur prior to stage 5 did indeed occur in the presence of α-amanitin, we counted the total number of tracer-labeled and unlabeled micromeres in the control and experimental embryos. The α-amanitin-injected embryos contained all five D’-derived micromeres and all ten of the primary quartet, secondary and tertiary trios of micromeres that are generated during stage 4 (Table 1). Thus, these early unequal cleavages do not require zygotically transcribed gene product(s) but rather are controlled by maternally provided gene product(s). The control embryos contained one to two additional unlabeled small cells because the primary quartet of micromeres had begun to divide (Sandig and Dohle, 1988). Thus, α-amanitin does block the subsequent divisions of the micromeres, as suggested earlier (Bissen and Weisblat, 1991).

Unequal cell divisions continue throughout later stages of leech development. For example, each teloblast undergoes a repetitive series of highly unequal divisions during stages 5-7 to generate a longitudinal chain or bandlet of small primary blast cells. These highly unequal blast cell-forming divisions continue, at a slightly reduced rate, in stage 7 embryos that have been injected with α-amanitin (Bissen and Weisblat, 1991). Although these blast cell-forming divisions do not require newly transcribed gene products, it is possible that they do require zygotic transcripts that were produced at an earlier stage of development. To test this hypothesis, we blocked transcription from the start of development and determined whether the supernumerary D’-derived yolk-rich cells were able to undergo teloblast-like divisions and form chains of small cells.

To do this, we injected stage 1 embryos with α-amanitin and injected the ectodermal precursor (DNOPQ) and the mesodermal precursor (DM) with lineage tracers of different colors at stage 4b. The embryos were fixed at 52 hours AED (mid stage 7), and the number of tracer-labeled yolk-rich cells and bandlets of small cells were counted in both groups of embryos. As expected, the control embryos (n=5) contained eight DNOPQ-derived teloblasts and bandlets and two DM-derived teloblasts and bandlets. The α-amanitin-injected embryos (n=8), on the other hand, contained from 11 to 15 DNOPQ-derived yolk-rich cells (av = 13.3±1.5), of which zero to four had generated bandlets of small cells (av = 2.4±1.5). The experimental embryos also contained from five to eight DM-derived yolk-rich cells (av = 6.0±1.3), of which zero to three had generated bandlets of small cells (av = 2.2±1.2).

These data indicate that some of the supernumerary D’-derived yolk-rich cells in the α-amanitin-injected embryos were able to undergo teloblast-like divisions and generate bandlets of small cells, as shown in Fig. 2C. Since transcription was blocked in these embryos from the start, it appears that these unequal blast cell-forming divisions proceeded in the absence of any zygotically transcribed gene products. Although some of the supernumerary D’-derived cells in the α-amanitin-injected embryos were able to generate bandlets of cells, these embryos were far from normal. The average number of bandlets in the experimental embryos was only about 40% of the control embryos, the experimental bandlets contained fewer cells than the control bandlets and the experimental bandlets were positioned randomly within the embryos. Since the divisions of the blast cells are blocked in α-amanitin-treated embryos (Bissen and Weisblat, 1991), these small cells never divided and the experimental embryos ceased to develop. Nevertheless, the fact that any of the D’-

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**Table 1. Number of cells in control and α-amanitin-injected embryos***

<table>
<thead>
<tr>
<th>Time†</th>
<th>Condition</th>
<th>D’-derived yolk-rich cells</th>
<th>D’-derived micromeres</th>
<th>D’-derived blast cells</th>
<th>Total D’-derived cells</th>
<th>Unlabeled micromeres</th>
<th>Unlabeled macromeres</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Control (n=14)</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>11</td>
<td>11±1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>α-amanitin (n=17)</td>
<td>5.4±0.9</td>
<td>5</td>
<td>0.1±0.3‡</td>
<td>10.5±0.8</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>Control (n=8)</td>
<td>4</td>
<td>7</td>
<td>4.8±1.0§</td>
<td>15.8±1.0</td>
<td>12±1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>α-amanitin (n=14)</td>
<td>12.2±2.3</td>
<td>5</td>
<td>0</td>
<td>17.2±2.3</td>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>

*Data are from four separate experiments. Presented are mean number of cells ± s.d. When all the embryos contained the same number of cells, the s.d. (0) is not presented.
†Hours after egg deposition (AED).
‡Two of the 17 embryos each contained one blast cell.
§The embryos had either 4 or 6 m blast cells.
¶These embryos contain more cells because micromere d’ had divided at 14 hours and micromere c’ had divided at 16 hours.
derived cells in the α-amanitin-injected embryos were able to
divide unequally and generate bandlets of small cells suggests
that this type of unequal cell division does not depend upon
zygotically transcribed gene products.

These data also indicate that the earlier α-amanitin-induced
conversions of unequal cleavages to equal cleavages did not
change the fate of all of the D'-derived cells to that of
teloblasts. In fact, the number of D'-derived cells that
underwent teloblast-like divisions was less than normal. It is
impossible, however, to determine whether those that did not
undergo teloblast-like divisions had assumed the alternate fate
(i.e., that of micromeres), since the divisions of the normal
micromeres are blocked by transcriptional inhibitors (see
above). Nonetheless, it appears that the unequal cleavages of
the large cells of the D' lineage during stage 5 have an absolute
requirement for zygotic transcription, whereas the earlier
micromere-forming and the later blast cell-forming unequal
cleavages do not.

Zygotically transcribed gene product(s) are required
for correct spindle orientation in the large cells of
the D' lineage

The symmetry of cell division is determined by the placement
of the cleavage furrow, which in turn depends upon the
location of the mitotic spindle or spindle asters (reviewed by
Rappaport, 1986). In unequally dividing cells, the mitotic
spindles are located eccentrically and the cleavage furrows are
placed asymmetrically. In leech embryos, the nuclei of the
yolk-rich D'-derived cells are located near one pole of the cell,
and the mitotic spindles are assembled at this peripheral
location. Fig. 3 presents two yolk-rich D'-derived cells in
which the condensed chromosomes and mitotic spindles lie
within the domain of yolk-deficient cytoplasm at one side of
each cell. Both of these cells were in anaphase at the time of
fixation and the orientation of their mitotic spindles can be
inferred from the locations of the Hoechst-stained separating
sister chromatids (arrows), which had been moving toward the
poles of the spindles.

In cell NOPQ of the control embryo shown in Fig. 3A, the
anaphase mitotic spindle was aligned with the radial axis of the
cell and was oriented orthogonal to the plasma membrane, with
one pole closely abutting the cell cortex. Since the cleavage
furrow bisects the spindle, two cells of drastically different
sizes would have been produced. In the yolk-rich D'-derived
cell of the α-amanitin-injected embryo shown in Fig. 3B,
however, the mitotic spindle was perpendicular to the radial
axis of the cell, as indicated by the positions of the separating
anaphase chromatids (arrows). Since the spindle was not
oriented orthogonally to the cell membrane, an equal cleavage
would have ensued. There were eight D'-derived cells in the
α-amanitin-treated embryos that were in metaphase or
anaphase at the time of fixation, and all of their condensed
chromosomes/chromatids were aligned like those shown in
Fig. 3B. These data suggest that zygotically transcribed gene
product(s) are involved in positioning the mitotic spindles of
the large cells of the D' lineage for their unequal cleavages
during stage 5. For these cleavages, the axis of the spindle must
be orthogonal to the cell membrane with the peripheral pole
close to the cell cortex. In the absence of zygotic transcription,
the mitotic apparatus does not assume the proper orientation
and these unequal cleavages are converted to equal cleavages.

DISCUSSION

We are interested in understanding how the very regular,
highly patterned cell divisions of leech embryos are
regulated. Previously we showed that the cleavages prior to
stage 5 are under maternal control, since their timing,
symmetry and orientation proceed normally in the presence
of α-amanitin (Bissen and Weisblat, 1991). Although the
transition to zygotic control of cell division begins around

Fig. 3. Zygotically transcribed
gene products are essential for
correct spindle orientation in the
large cells of the D' lineage.
Digitally deconvolved images of
sections of Hoechst-stained
embryos fixed at 16 hours AED.
The orientation of the mitotic
spindles can be inferred from
the locations of the Hoechst-
stained separating sister
chromatids (arrows), which had
been moving toward the poles
of the spindles at the time of
fixation. (A) Control embryo in
which the left NOPQ' 
proteloblast was in anaphase.
The spindle was oriented
orthogonal to the plasma
membrane, in anticipation of an
unequal cell division (to produce a nopq'' micromere). Part of the right NOPQ' proteloblast is visible in the upper right corner. (B) α-amanitin-
injected embryo in which one of the supernumerary D'-derived yolk-rich cells was in anaphase. The spindle was not oriented orthogonal to the
plasma membrane but rather was aligned with the long axis of the cell. As a result of this orientation, an equal cell division would have ensued.
The small yolk-deficient cell located above and to the left of the large anaphase cell is a DM-derived micromere that was generated during
stage 4. Bar, 50 μm.
stage 5, it occurs in a cell-specific and lineage-specific manner. The progression of the cell cycle in some cells depends upon the products of zygotic transcription. For example, the divisions of the micromeres and the blast cells are blocked after the inhibition of transcription, but the divisions of the teloblasts continue quite normally in the presence of a transcriptional inhibitor (Bissen and Weisblat, 1991). Furthermore, we know that the micromeres and the blast cells transcribe cdc25, which encodes a protein phosphatase involved in the initiation of mitosis, whereas the early blastomeres, macromeres and teloblasts contain maternal cdc25 mRNA (Bissen, 1995).

Here we describe a second requirement for zygotic transcription, namely that zygotically transcribed gene products are required to direct the symmetry of division of the large cells of the D’ lineage during stage 5. In the absence of zygotic transcription, these cells undergo equal cleavages, rather than following their normal pattern of equal and unequal cleavages. Presumably their rate of cell division is not altered by transcription, these cells undergo equal cleavages, rather than following their normal pattern of equal and unequal cleavages. Thus, it appears that zygotically transcribed gene product(s) are required to establish or maintain the orthogonal orientation of the mitotic spindles required for the unequal cleavages of the large cells of the D’ lineage during stage 5.

Spindle orientation is determined by the location of the spindle pole asters, which emanate from the centrosomes. At the beginning of mitosis the duplicated centrosomes separate and migrate to opposite sides of the nucleus to form the two poles of the mitotic spindle (Mazia, 1987). During each successive cell cycle the axis of migration of the centrosomes shifts 90° relative to the previous axis of migration. This predictable migration of the centrosomes leads to a default pattern of cell division in which each division occurs at right angles to the preceding division. This pattern of alternating divisions is common during the early development of invertebrate embryos (Wilson, 1925). To deviate from this pattern an additional mechanism is required to reposition the centrosomes. For example, the centrosomes and nucleus of each P blastomere of C. elegans embryos undergo an additional 90° rotation, which allows these cells to divide successively along the same axis (Hyman and White, 1987).

During the early stages of leech development the cell divisions follow the default mode in which the axis of the spindle is shifted 90° relative to that of the previous spindle. Around the start of stage 5, however, the spindles of some of the yolk-rich D’-derived cells are perturbed in the majority of the α-amanitin-injected embryos, aberrant divisions were observed 1-2 hours before the start of stage 5 (Bissen and Weisblat, 1991). Thus, the unequal division of DNOPQ” (whose spindle orientation shifts by 45°) can also be perturbed by transcriptional inhibition. Diameter of an embryo is approximately 400 μm.

Fig. 4. Schematic illustration depicting the divisions of the large cells of the DNOPQ lineage. Development progresses from upper left to lower right. An early stage 5 embryo is illustrated in the upper right drawing and an early stage 6 embryo is presented in the lower right drawing. Views are from the animal pole. In each drawing, the orientation of the spindle of each dividing cell is designated by a short thick line, while the axis of the spindle of the previous division(s) is denoted by a dotted line. The shifts in spindle orientation (90° or 45°) are indicated for each division. The divisions that are sensitive to α-amanitin are those in which the orientation of the spindle shifts by 45° (i.e., those of the NOPQ cells). While the cleavages of the large D’-derived cells are perturbed in the majority of α-amanitin-injected embryos after the start of stage 5, the onset of α-amanitin sensitivity is not absolute. In approximately 13% of the α-
the D'-derived blastomeres undergo 45° shifts in orientation rather than 90° shifts (Sandig and Dohle, 1988), as diagrammed in Fig. 4. The cells that display these 45° shifts in spindle orientation (i.e., the NOPQ-derived cells) are some of the cells whose divisions during stage 5 are perturbed by the inhibition of zygotic transcription.

The centrosomes of the yolk-rich D'-derived cells of the α-amanitin-treated embryos apparently migrate to the opposite sides of the nuclei, since bipolar mitotic spindles are formed and these cells are able to divide. However, their spindles are not oriented orthogonal to the cell membrane, suggesting that the centrosomes are unable to rotate to new positions. Thus, we hypothesize that zygotically transcribed gene product(s) may be required to rotate the centrosomes so that the mitotic spindles undergo 45°, rather than 90°, shifts in orientation between these divisions. The altered cleavages of these D'-derived cells in the α-amanitin-treated leech embryos are similar to those of the vegetal blastomeres of detergent-treated sea urchin embryos. Detergent treatment causes the vegetal blastomeres to cleave equally rather than unequally (Tanaka, 1976) because the spindles of the vegetal blastomeres fail to rotate and are oriented horizontally rather than vertically in the sea urchin embryos (Dan, 1979).

Since the teloblasts of leech embryos divide repeatedly along the same axis when producing primary blast cells, we presume their centrosomes undergo additional rotations, similar to those of the P cells of C. elegans (Hyman and White, 1987). The repeated unequal divisions of the teloblasts do not require zygotically transcribed gene products, whereas the large D'-derived cells do require zygotically transcribed products for their unequal cleavages during stage 5. Thus, there must be some difference in the mechanisms of centrosome rotation or spindle positioning between the teloblasts and their precursor cells (the large D'-derived cells of the stage 5 embryo).

The final position of the mitotic spindle also depends upon physical interactions between the peripheral spindle pole aster and the cortex of the cell. Centrifugation and micromanipulation experiments have revealed that the positioning of the spindle is aided by the peripheral aster establishing a strong linkage to a specific position on the cortex (Conklin, 1917; Kawamura, 1977; Lutz et al., 1988). It has been observed in Spisula eggs that the metaphase spindle rocks back and forth until ‘contact’ is established between the peripheral aster and a site on the cortex (Dan and Inoue, 1987). In C. elegans both of the centrosomes in the P cells initiate rotation towards a defined region of the anterior cortex until microtubule-mediated contact is established by one (Hyman, 1989). A spindle pole that is closely apposed to the cortex displays a flattened or truncated aster (Czihak, 1973; Kawamura, 1977; Dan, 1979; Dan and Inoue, 1987; Schroeder, 1987; Lutz et al., 1988), as well as a smaller and elongated centrosome (Holy and Schatten, 1991). Spindles isolated after attachment to the cortex have a detergent-resistant membrane component associated with the truncated aster (Dan and Ito, 1984). Thus, since attachment of the peripheral spindle pole to a defined site on the cell cortex seems critical for the proper positioning of an asymmetrically located spindle, it is possible that zygotically transcribed gene product(s) are required in the large cells of the D’ lineage of stage 5 leech embryos for moving the spindle pole to the cortex or for anchoring it to the cortex.

In summary, the products of zygotic transcription are required in the large cells of the D’ lineage to establish or maintain an orthogonal spindle orientation for the unequal cleavages that occur during stage 5. These gene products could be involved in any aspect of the interactions between the cytoskeleton, the centrosomes and the cortex of these D’-derived blastomeres. We are currently seeking to identify these zygotically transcribed gene product(s) and elucidate their role(s) in these unequal cleavages.

This work was supported in part by a Basil O’Connor Research Grant (No. 5-1140) from the March of Dimes Birth Defects Foundation. We thank David Weisblat for helpful suggestions on the manuscript.

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(Accepted 27 October 1995)