Nuclear position in the cells of the mouse early embryo

By W. J. D. Reeve 1 and F. P. Kelly 2

From the Department of Anatomy and the Statistical Laboratory, Cambridge

SUMMARY

The nuclei of preimplantation mouse embryos were identified after labelling with either DAPI or Hoechst 33258. During the 4- and 8-cell stages the peripherally located nuclei become clustered nearer the centre of the embryo. This nuclear migration towards the base of each cell was also observed during the development of couplets of 2/4 and 2/8 cells. Most blastomeres isolated from compact 8-cell embryos contained a nucleus located in their basal half away from the microvillous pole. The displacement may be critical for the generation of a range of sizes of blastomeres in the 16-cell embryo.

INTRODUCTION

The 8-cell preimplantation mouse embryo undergoes a process of compaction in which increased intercellular apposition prevents the ready identification of individual cells (Lewis & Wright, 1935; Ducibella, 1977). At compaction, each cell of the 8-cell embryo also becomes polarized, and displays several features typical of an epithelial cell (Johnson, Pratt & Handyside, 1981; Johnson, 1981a). The polarization that occurs in the individual blastomeres of the 8-cell embryo is revealed by the restriction of surface fluorescent-ligand binding sites (Handyside, 1980; Ziomek & Johnson, 1980; Johnson & Calarco, 1980) and microvilli (Ducibella, Ukena, Karnovsky & Anderson, 1977; Reeve & Ziomek, 1981) predominantly to the apical, normally outward-facing surface of each cell, alkaline phosphatase activity confined to apposed cell surfaces (Johnson, Calarco & Siebert, 1977; Mulnard & Huygens, 1978; Izquierdo, Lopez & Martinez, 1980), the restricted cytoplasmic localization of endocytic vesicles containing horseradish peroxidase (HRP) peripheral to the nucleus of each cell (Reeve, 1981a), and the high incidence of mitochondria and microtubules located near areas of cellular apposition (Ducibella et al., 1977). In this paper, individual cells of the 8-cell embryo are shown to develop another feature that is characteristic of an epithelial cell, namely a nuclear position at the base of the cell away from the apical pole of microvilli. Moreover, it is shown that this basal position may be critical for the generation of blastomeres of different sizes in the 16-cell embryo (Johnson & Ziomek, 1981; Reeve, 1981b).

1 Author's address: Department of Anatomy, Downing Street, Cambridge, CB2 3DY, U.K.
2 Author's address: Statistical Laboratory, 16 Mill Lane, Cambridge, CB2 1SB, U.K.
MATERIALS AND METHODS

Embryo collection

Female HC-CFLP mice, 3–4 weeks old, (Hacking & Churchill Ltd., Alconbury) were induced to superovulate with intraperitoneal injections of 5 i.u. of pregnant mare's serum gonadotrophin (PMS: Folligon, Intervet), followed after 44–48 h by 5 i.u. of human chorionic gonadotrophin (hCG: Chorulon, Intervet). Females were paired with HC-CFLP males, and vaginal plugs taken as an indication of mating.

Embryos were flushed from the oviducts with phosphate-buffered medium 1 supplemented with 0·4 % (w/v) bovine serum albumin (PB1 + BSA; Whittingham & Wales, 1969) and cultured at 37 °C in medium 16 with 0·4 % (w/v) BSA (M16 + BSA; Whittingham, 1971) in 5 % CO₂ in air. 4-cell embryos were harvested at hourly intervals from cultures of 2-cell embryos that were flushed at 46 h post-hCG; newly formed, precompact 8-cell embryos were harvested at hourly intervals from cultures of 4-cell embryos that were flushed at 58 h post-hCG; compact 8-cell embryos were selected either from a population of embryos that was flushed at 68 h post-hCG, or from 8-cell embryos that compacted in vitro. Zona pellucidae were removed by a 15 to 30 s incubation in prewarmed (37 °C) acid Tyrode's solution (pH 2·5) + 0·4 % (w/v) polyvinylpyrrolidone (Nicolson, Yanagimachi & Yanagimachi, 1975).

Decompaction and disaggregation

Compact embryos were decompacted by incubation for 20–30 min in calcium-free medium 16 + 0·6 % (w/v) BSA (Pratt, Ziomek, Reeve & Johnson, 1982). Embryos were disaggregated to single cells by pipetting with a flame-polished micropipette.

Terminology

Throughout this paper, single blastomeres from 2-cell, 4-cell and 8-cell embryos are called 1/2, 1/4 and 1/8, respectively. Natural 2/4, 2/8 and 2/16 couplets were harvested at hourly intervals from populations of 1/2, 1/4 and 1/8 cells, respectively, cultured to divide in vitro.

Fixation

Embryos and isolated cells were fixed in 4 % (w/v) paraformaldehyde (Anderson & Co. Ltd.) in phosphate-buffered saline (PBS) at room temperature for 1 h, before washing and storage in PB1 + BSA at 4 °C.

Fluorescent labelling

(a) Fluorescein-conjugated concanavalin A.

Fluorescein-conjugated concanavalin A (FITC-Con A; Miles Labs) was used...
Nuclear position in the mouse embryo

119
to label the position of the microvillous pole on isolated cells (Handyside, 1980; Reeve & Ziomek, 1981). Blastomeres were incubated for 15 min at room temperature in FITC-Con A (700 µg/ml in PB1 + BSA + 0.02% (w/v) sodium azide). Cells were washed thoroughly before fixation.

(b) **DAPI**

Embryos were stained vitally by incubation in 10 µg/ml 4', 6-diamidino-2-phenylindole (DAPI; Sigma; Williamson & Fennell, 1975) in M16 + BSA at 37°C in 5% CO2 in air for 1–3 h. The fluorescent staining of the nuclei was observed immediately. 8-cell embryos do not form blastocysts when cultured continuously in 10 µg/ml DAPI, but a pulse of 3 h did not prevent subsequent blastocyst formation.

(c) **Hoechst 33258**

Nuclear DNA was stained by incubating fixed embryos and isolated cells in 50 µg/ml Hoechst 33258 (gift of Dr Brigid Hogan) in PB1 + BSA for 1–5 h at room temperature (Hilwig & Gropp, 1972). Generally, nuclear position was revealed after staining of fixed blastomeres with Hoechst 33258. Labelling with DAPI occurred only when the locations of nuclei in fixed material were compared with those for living material (Tables 1 and 3).

**Fluorescence microscopy and photography**

Labelled embryos and cells were washed extensively, and examined in wells of a tissue-typing slide (Baird & Tatlock) in drops of PB1 + BSA under oil. A Zeiss Universal microscope, which was fitted with incident source HBO 200 and IIIRS condenser, was used to examine cells: Zeiss filter set 487709 (plus additional excitation filter LP 425) was used to examine cells for FITC-labelling; Zeiss filter set 487705 allowed the examination of the nuclei labelled with either Hoechst 33258 or DAPI. Photomicrographs were taken on Kodak Tri-X 35 mm film; all fluorescence exposures were for 2 min. Most measurements of the distribution of the nuclei in intact embryos were obtained from adjacent bright-field and fluorescence negatives. However, for the examination of the location of the nucleus in an isolated cell, the fluorescence photomicrograph was superimposed on a bright-field photomicrograph of exactly the same area. An exposure time of 1/30 the light-metre reading gave a negative of reduced density that allowed observation of the nucleus and the outline of the cell when the negatives were superimposed. All measurements were recorded from images of photographic negatives projected on a Zeiss Documentor microfilm reader model DL II. Any fixed cells that showed disrupted cell membranes were excluded from the data, in case eruption of cytoplasmic contents had affected nuclear position.
RESULTS

Intact embryos

The distribution of nuclei within intact 4- and 8-cell embryos was estimated, as illustrated in Figs 1–4, by measurement of the minimum diameter to enclose all cells (A) and the minimum diameter to enclose all nuclei (B), and thus deriving the ‘clustering ratio’ $\frac{A-B}{A}$ (Table 1).

Fixed early and late 4-cell embryos differed significantly in the clustering of

Fig. 1. A precompact 8-cell embryo fixed and then stained with Hoechst 33258 and viewed by fluorescence microscopy. The nuclei are located peripherally.

Fig. 2. A decompacted late 8-cell embryo fixed and then stained with Hoechst 33258. The nuclei are clustered to the centre of the embryo.

Fig. 3. Representation of nuclei in cells of 8-cell embryo. The measurements A and B were recorded for each embryo, and used to calculate $\frac{A-B}{A}$, the clustering ratio.

Fig. 4. A decompacted 8-cell embryo stained vitally with DAPI and viewed by fluorescence microscopy to reveal the nuclei located towards the centre of the embryo.
Table 1. *Nuclear position in intact embryos as assessed by staining with Hoechst 33258* or DAPI**.

<table>
<thead>
<tr>
<th>Stage of embryo</th>
<th>Treatment</th>
<th>Number of embryos</th>
<th>Mean diameter of embryos ± s.d.</th>
<th>Mean A-B*** ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-cell</td>
<td>early (0–1 h)</td>
<td>28*</td>
<td>19.7 ± 1.5</td>
<td>0.22 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>late (8–9 h)</td>
<td>31*</td>
<td>19.4 ± 1.1</td>
<td>0.31 ± 0.09</td>
</tr>
<tr>
<td>8-cell</td>
<td>precompact</td>
<td>32*</td>
<td>18.1 ± 1.1</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>precompact + Ca\textsuperscript{2+}-free</td>
<td>33*</td>
<td>17.9 ± 1.1</td>
<td>0.11 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>compact</td>
<td>23*</td>
<td>17.4 ± 1.2</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>decom pact</td>
<td>26*</td>
<td>18.3 ± 0.8</td>
<td>0.25 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>precompact</td>
<td>24**</td>
<td>19.1 ± 1.1</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>decom pact</td>
<td>16**</td>
<td>18.5 ± 0.7</td>
<td>0.20 ± 0.04</td>
</tr>
</tbody>
</table>

*** A-B = maximum diameter of embryo – minimum diameter to enclose all nuclei (see Fig. 3)
their nuclei within the embryo (at the 1% level, using a two-sample t-test). Precompact 8-cell embryos (Fig. 1) had low values of $A^2B^2$, indicating a relatively peripheral position of the nuclei, whereas compact 8-cell embryos had more clustered nuclei. (The mean values for precompact and compact embryos are significantly different at the 1% level, using a two-sample t-test.) This difference in nuclear clustering did not depend on the compacted embryo containing flattened cells since it was also apparent after decompaction following exposure of precompact and compact (Fig. 2) embryos to Ca$^{2+}$-depleted medium. In fact, the nuclei of decompacted embryos appeared even more clustered than those of compact embryos, due presumably to the greater intercellular apposition and reduced mean diameter of compact embryos (Table 1). The difference in nuclear distribution was not induced by fixation since the increased clustering of the nuclei in decompacted embryos was also demonstrated after vital staining with DAPI (Fig. 4; Table 1).

Isolated cells

Late 1/8 cells are polarized. It is therefore possible to determine the relationship between nuclear position and the orientation of surface polarity in isolated 1/8 cells (Fig. 5). 4-cell embryos were allowed to divide to 8-cell embryos that were then cultured in individual microdrops and assessed visually for the onset of compaction. Soon after compaction, approximately 6–7 h after formation of the 8-cell embryo, embryos were disaggregated to single cells that were either fixed immediately or cultured for a further 2 h as single cells before fixation (Table 2; lines 1 & 2). The blastomeres analysed immediately after disaggregation showed a significant incidence of non-polar patterns of surface labelling, and of the polar cells 69% had the nucleus located in the half of the cell near the pole. In contrast, isolated blastomeres that were cultured for a further 2 h had an increased incidence of polarity, and in most (56%) blastomeres the nucleus was located away from the pole. When blastomeres were retained in the embryo in situ for this 2 h period, and then disaggregated, 65% of the polar cells had the nucleus sited away from the apical fluorescent pole (Table 2; line 3). The basal nuclear location is not an artifact of fixation, since the distribution of nuclei appears similar for isolated cells stained vitally with DAPI and then stained again, postfixation, with Hoechst 33258 (Table 3). In a subsequent experiment.

Fig. 5. An isolated 1/8 blastomere was surface-labelled with FITC-Con A, and fixed before labelling of the nucleus with Hoechst 33258. (A) The fluorescent image of the nucleus (Hoechst 33258) was superimposed on a brief bright-field exposure of the cell. (B) Usually, the pole of ligand binding (FITC-Con A) was located away from the nucleus. 

Fig. 6. The positions of the nuclei were identified in 2/8 natural couplets that had been fixed and labelled with Hoechst 33258. (A) In newly formed couplets, the nuclei tend to be displaced maximally from the point of contact between the two blastomeres. (B) In late couplets (8–9 h), the two nuclei are closer than in early couplets.
Nuclear position in the mouse embryo

Figs 5 & 6
Table 2. Nuclear position in 1/8 cells isolated from 8-cell embryos as revealed by staining fixed blastomeres with Hoechst 33258.

<table>
<thead>
<tr>
<th>Time of disaggregation (h after formation of 8-cell embryo)</th>
<th>Time of fixation (h after disaggregation)</th>
<th>Total No.</th>
<th>Polar*</th>
<th>Non-polar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Near pole (%)</td>
<td>Away from pole (%)</td>
</tr>
<tr>
<td>Immediately after compaction (~6–7 h)</td>
<td>0</td>
<td>48</td>
<td>18 (69)</td>
<td>6 (23)</td>
</tr>
<tr>
<td>Immediately after compaction (~6–7 h)</td>
<td>2</td>
<td>54</td>
<td>16 (41)</td>
<td>22 (56)</td>
</tr>
<tr>
<td>Compaction + 2 h (~8–9 h)</td>
<td>0</td>
<td>40</td>
<td>8 (31)</td>
<td>17 (65)</td>
</tr>
</tbody>
</table>

*The centre of the nucleus of each polar cell was identified as being in the half of the cell near the apical pole, in the half away from the pole, or equidistant between the pole and the opposite, basal surface.

†Lost, damaged or unscoreable.
Table 3. Nuclear position in blastomeres of late 8-cell embryos stained first with DAPI and then with Hoechst 33258*

<table>
<thead>
<tr>
<th>Nuclear position revealed by DAPI (number of cells)</th>
<th>Nuclear position revealed by Hoechst dye† (number of cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near pole (3)</td>
<td>Near pole</td>
</tr>
<tr>
<td>Away from pole (37)</td>
<td>2</td>
</tr>
<tr>
<td>Equidistant (8)</td>
<td>0</td>
</tr>
<tr>
<td>Non-polar cells (4)</td>
<td>1</td>
</tr>
</tbody>
</table>

* Embryos were incubated in DAPI for 3 h before disaggregation and labelling with FITC-Con A. The positions of the nuclei were identified, and blastomeres sorted accordingly. After fixation, the three classes of blastomeres were stained separately with Hoechst 33258.
† The centre of the nucleus of each polar cell was identified as being in the half of the cell near the apical pole, in the half away from the pole, or equidistant between the pole and the opposite, basal surface.
‡ Lost, damaged or unscoreable.

in which the blastomeres of compact 8-cell embryos were either labelled with DAPI before disaggregation or, alternatively, isolated, fixed and then stained with Hoechst 33258 the incidence of basal nuclei was higher. Of 25 cells that were stained vitally with DAPI, and 25 cells stained postfixation with Hoechst 33258, 76 % and 80 %, respectively, had the centre of the nucleus located in the basal half of the cell.

2/4 and 2/8 couplets

The positions of the nuclei in 2/4 and 2/8 couplets varied with time after the division in vitro of isolated 1/2 and 1/4 cells, respectively. When the couplets were examined immediately after their formation (0–1 h) the nucleus of each cell was frequently displaced maximally from the point of contact between the two sibling cells (Fig. 6A; Table 4). At increasing times after formation (4–5 and 8–9 h), the nuclei of each couplet appeared less separated (Fig. 6B; Table 4), and stained more heavily than those of 0–1 h couplets due presumably to their increased content of DNA. The inward migration of the nuclei was more pronounced in the 2/8 than in the 2/4 couplets. The mean values after 0–1 h and 8–9 h are significantly different at the 5 % level for the 2/4 couplets, and at the 0.0001 % level for the 2/8 couplets, using two-sample t-tests. Of 38 late (8–9 h) 2/8 couplets (i.e. 76 blastomeres) that were decompacted in Ca²⁺-depleted medium before fixation, 27 blastomeres (36 %) had the nucleus located in the basal half near the point of cell contact, 45 (59 %) contained in apical nucleus and 4 (5 %) had the nucleus situated equidistant between the apex and base of the cell. Most of the nuclei of the 56 couplets that were examined within 1 h of
Table 4. The distribution of nuclei in 2/4 and 2/8 couplets formed by the division in vitro of isolated 1/2 and 1/4 cells, respectively. Couplets were fixed and stained with Hoechst 33258.

<table>
<thead>
<tr>
<th>Couplet</th>
<th>Time of examination (h after couplet formation)</th>
<th>Number of couplets</th>
<th>Nuclear separation (mean s/t* ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/4</td>
<td>0-1</td>
<td>27</td>
<td>0.79 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>4-5</td>
<td>36</td>
<td>0.73 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>8-9</td>
<td>47</td>
<td>0.74 ± 0.08</td>
</tr>
<tr>
<td>2/8</td>
<td>0-1</td>
<td>56</td>
<td>0.85 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>4-5</td>
<td>43</td>
<td>0.77 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>8-9</td>
<td>32</td>
<td>0.72 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>8-9 + Ca²⁺-free</td>
<td>38</td>
<td>0.75 ± 0.08</td>
</tr>
</tbody>
</table>

* See Fig. 6

formation were displaced maximally from the point of intercellular contact, and the nucleus was always located in the apical half of each cell.

Eccentricity of nuclear position in 1/8 cells is related to the relative cell sizes in 2/16 couplets

The position of the nucleus within a cell is variable about the geometrical centre of the cell, and is frequently further than 20% of the cell's radius from the centre. After a 1/8 cell divides, the daughter 1/16 cells frequently differ in volume by more than 30%. We have therefore considered the hypothesis that when a cell divides it does so about a randomly orientated plane through the nucleus, and that the differences in sizes of daughter cells can be explained as a consequence of the eccentricity of the nucleus in the parent cell.

Two sets of data are available. The first gives information on the position of the nucleus in a sample of 140 1/8 cells prior to division (Fig. 7). The second gives the sizes of the pairs of daughter cells after 128 cells of a different sample have divided (Fig. 8). In Section 1 of the appendix, two mathematical models for the hypothesis under consideration and the data sets available are described, and it is shown how the first data set can be used to predict the relative sizes of pairs of daughter cells under the hypothesis. In Section 3 of the appendix this prediction is compared with the second data set. The hypothesis is shown to be consistent with the two sets of data (Figs. 9 & 10).

DISCUSSION

Fluorescent labelling with Hoechst 33258 has been used previously to locate the nucleus within a cell (Berlin & Oliver, 1980). The photographic superimposition technique that has been introduced in this paper to identify the
Fig. 7. Measurements a, b, c and d were recorded for each 1/8 cell, thereby permitting calculation of the distance r of displacement of the centre of the nucleus from the centre of the cell, and the mean radius R of the blastomere.

Fig. 8. Measurements x, y, g and h were recorded for each 2/16 couplet, and were used to calculate the relative volumes of the two blastomeres in each couplet.
distribution of labelled nuclei within cells has several advantages over the alternative technique of obtaining measurements from an eyepiece graticule. The recording of all images on photographic negatives provides a permanent record of the subject material and allows more sensitive measurement. Furthermore, it
Nuclear position in the mouse embryo

is far easier to obtain measurements from the superimposed fluorescent and bright-field images on the same photographic negative than it is to do so when viewing a combination of the two down the microscope. One possible limitation could arise from a cell altering its position between the two exposures, but this is unlikely to remain undetected since a disturbed nucleus would be revealed by its indistinct outline and sometimes by its apparent position outside the cell.

During the lives of both 4-cell and 8-cell mouse embryos, the nuclei tend to become increasingly clustered to the centre of the embryo. The effect is less marked in 4-cell embryos. When examined soon after the formation of the 8-cell embryo, the nuclei are widely dispersed, and frequently appear to be distributed near the outward-facing surface of each cell. Subsequently, the increased clustering of nuclei in the later compacted embryo must be caused by a net movement of the nuclei towards the bases of individual cells. However, although the distributions of the nuclei differed significantly for fixed precompact and fixed decompacted embryos, this difference was less great when the nuclei were stained vitally. The differences in the positions of the nuclei between fixed and vitally stained embryos could result either from a fixation-induced migration of the nuclei to the bases of cells, or from a peripherally directed movement of the nuclei during the processing of the vitally stained embryos. Fixation may cause shrinkage of embryos (Table 1), and there is some evidence that when fixed cells are damaged their nuclei may be displaced. The photographic assessment of nuclear position necessarily exposed the vitally stained embryos to prolonged incubations in either PB1 + BSA or, to maintain decompaction, calcium-free medium + BSA on tissue-typing slides at room temperature. These treatments are likely to be harmful, and may indeed affect nuclear position, although culture of 8-cell embryos for 3 h in 10 μg/ml DAPI at 37 °C does not prevent blastocyst formation, and is itself therefore unlikely to have caused a redistribution of the nuclei. Despite these qualifications, the data may reasonably be interpreted as suggesting an inward movement of nuclei during the 8-cell stage.

The examination of 2/4 and 2/8 couplets at various times after their formation from isolated 1/2 and 1/4 cells, respectively, also suggests that the nuclei migrate away from the apices of cells and towards the region of contact between the two blastomeres. The level of migration was greater and more significant in the 2/8 than in the 2/4 couplets (Table 4). However, although the nuclei of 2/8 couplets appeared closer at increased times after the division, there was considerable variation in their positions and only a minority of blastomeres contained a nucleus that actually had its centre located in the basal half of the cell. There are two possible explanations for the variability: either there is a real variability in the intact embryo, or, alternatively, nuclear migration in the cells of couplets is not equivalent to that in the intact embryo, due presumably to modifying effects of intercellular contacts and apposition in the latter case. Support for a possible influence of cellular apposition on nuclear position arises from observations on cells both immediately after their isolation, and also after their culture in isolation.
for 2 h (Table 2, lines 2 & 3). Taken together, however, these results suggest that during the 8-cell stage nuclei tend to move in an apical to basal direction (i.e. to the centre of the embryo), and that this movement cannot be explained exclusively as a result of increased intercellular flattening. The data are consistent with the possibility that nuclear migration is a further feature of the polarization of cells occurring at this stage. The less marked tendency of nuclei to migrate basally observed in intact 4-cell embryos or in 2/4 couplets may indicate that the migration of nuclei at the 8-cell stage is an exaggeration of a more general, cell-cycle related phenomenon. Alternatively, it could indicate that the cell interaction that irreversibly induces polarity at the 8-cell stage is already effective but reversible at earlier stages. The notion that the generation of polarity might develop progressively, and reversibly, during earlier cleavage stages, and some evidence to support it, has been suggested previously (Johnson, 1981b).

The mitotic apparatus, and especially the asters, acts as the stimulus for the position and orientation of the cleavage furrow (Wolpert, 1960; Rappaport, 1974). The cleavage plane is established in the plane previously occupied by the metaphase plate, and usually bisects the metaphase spindle. Furthermore, the manipulation of the mitotic apparatus relative to the cell surface induces a new furrow in the surface adjacent to the asters in the new position (Conklin, 1917; Clement, 1935). Eccentric division is indeed associated with the displacement of the mitotic apparatus from a central position, and is typical of many embryos (Balinsky, 1975; Berrill & Karp, 1976). The transition from an 8- to a 16-cell mouse embryo involves the generation of two subpopulations of cells of different relative sizes (Johnson & Ziomek, 1981). Generally, a polar 1/8 cell divides to form a larger, polar 1/16 cell and a smaller, apolar 1/16 cell, and this occurs whether division of cells occurs in situ or after isolation in vitro. The larger cells occupy an outer position in the morula and have properties that anticipate trophectoderm; the smaller cells occupy an inner position, and are ICM-like (Handyside, 1981; Ziomek & Johnson, 1981). To what extent can the differences in size of 1/16 cells of each subpopulation be explained by the basal displacement of the nuclei in the polarized 1/8 cells?

The hypothesis that a 1/8 cell divides about a randomly orientated plane through the nucleus, and that the displacement of the nucleus causes the disparity in size between the two daughter cells, was not disproved experimentally. Such divisions could account for the range of relative sizes of the two daughter cells of 2/16 couplets. Moreover, this range cannot arise from a consistent, preferred orientation of the cleavage plane through the nucleus, such as across the longest or shortest axes of the cell. In this regard, the division of isolated 1/8 cells to 2/16 couplets does not appear to adhere strictly to Hertwig’s (1899) notion that the metaphase plate of a dividing cell becomes orientated orthogonally to the long axis of the cell. It has been claimed that this ‘rule’ may apply to the early dividing blastomeres of the mouse 8-cell embryo (unpublished observations of Graham, cited by Graham & Kelly, 1977), but both the data on the
relative sizes of blastomeres in natural 2/16 couplets and the significant incidence of division through the microvillous pole (see later) argue against such a preferred plane of cleavage at division of 1/8 to 2/16 cells. However, this argument is best settled by an analysis of the orientation of the metaphase plates in dividing blastomeres in the embryo in situ. It is possible that Hertwig’s rule may apply to the 16-cell embryo and later developmental stages in which there is a strong tendency for the flattened outside cells to ‘breed true’ and produce only other outside cells (Johnson & Ziomek, 1983).

Several assumptions and sources of error will undoubtedly have reduced the closeness of the correlation between nuclear position in 1/8 cells and the relative cell sizes in 2/16 couplets. First, the shapes of all 1/8 and 1/16 cells may not be represented exactly by perfect spheres and ellipsoids, respectively. This applies especially to the blastomeres of 2/16 couplets, which, even after incubation in Ca^{2+}-free medium, showed very variable amounts of intercellular apposition. Second, the nucleus has been identified by a point at its centre, and possible variations in nuclear shape and size have been ignored. One source of error may arise from the examination of damaged cells in which the nuclei have been displaced artificially. Furthermore, more serious errors might be caused by any preferential orientation of polarized 1/8 cells during measurement of nuclear eccentricity. The nuclear distribution did not differ for unlabelled cells and those labelled with fluorescent ligand, and so it is unlikely that the binding of Con A results in a preferential orientation that biases the data. Nonetheless, if a cell never settled on its microvillous pole (Reeve & Ziomek, 1981), and if the nucleus was always located on a line passing through the pole and the centre of the cell, the projected distance of the nucleus from the cell centre would appear greater than if the cell had a random orientation. However, it is difficult to assess the possible magnitude of this error since the position of the nucleus relative to both the pole and the centre of the cell was variable.

The position of the nucleus in 1/8 blastomeres can also be related to the incidence of the different phenotypes of blastomeres in 2/16 couplets (see Reeve, 1981c). Thus, the incidence of division through the microvillous pole (26 out of 146 couplets (18 %)) provides additional evidence against a consistent plane of division orthogonal to the long axis of the blastomere. Secondly, however, division in the plane of the nucleus in 1/8 blastomeres does not appear to be consistent with the correlations of polarity and relative cell sizes that are observed typically between the two blastomeres of individual 2/16 couplets. Thus, of 140 late 1/8 cells examined for nuclear position, 68 were either not examined for surface-labelling patterns or had a ring pattern of surface labelling. Of the remaining 72 cells, for which polarized patterns of labelling were recorded, 54 (75 %) had the nucleus sited basally and 18 (25 %) had the nucleus located in the polar half of the cell. In contrast, in a previous examination of 146 FITC-labelled 2/16 couplets, only 4 couplets (3 %) were identified as containing a larger, apolar cell and a smaller, polar cell, whereas 100 couplets (68 %) had
a larger, polar cell and a smaller, apolar cell (Reeve, 1981c). These two classes of couplets were formed presumably from 1/8 cells with apical and basal nuclei, respectively. The other 42 couplets (29 %) contained cells that were both polar and of similar sizes, both polar and of different sizes, or that could not be distinguished by size, only one cell being polar. (Comparative cell size was determined by actual observation, rather than by precise measurement.) It is difficult to predict the location of the nuclei in the 1/8 blastomeres that generated these 42 couplets. However, certain points are relevant. First, a 1/8 cell with an apical nucleus is more likely than a 1/8 blastomere with a basal nucleus to generate a couplet containing two polar cells, since the incidence of bisection of the microvillous pole will increase with the increased proximity of the nucleus to the pole. Second, when the majority of 1/8 blastomeres possess basal nuclei, the representation of a three-dimensional object in a two-dimensional projection will ensure that some nuclei that are in fact basal appear, in the two-dimensional image, to have their centre in the apical half of the cell. Thus, the correlations between polarity and relative cell size in 2/16 couplets may be compatible with predictions of the relative sizes of blastomeres in 2/16 couplets based on the distances of displacement of the nuclei from the centres of individual 1/8 cells.

In conclusion, the data presented here are consistent with a basal net movement of nuclei in blastomeres of the compacted 8-cell mouse embryo, and with an effect of this basal displacement on the relative sizes of the daughter cells at the subsequent mitotic division.

We thank Drs Martin Johnson, Carol Ziomek, Hester Pratt, Harry Goodall and Hilary MacQueen for valuable discussion and criticism, Dr Brigid Hogan for a gift of Hoechst 33258, Jackie Kelly for programming the numerical integration procedure, and John Bashford, Gin Flach, Mike Parr and Ian Edgar for technical help. This work was supported by grants from the Medical Research Council, the Ford Foundation and the Cancer Research Campaign to Dr M. H. Johnson.

REFERENCES


Nuclear position in the mouse embryo


Appendix

1. Mathematical models

The cell is idealised as a sphere, and the nucleus as a dimensionless point. The hypothesis is that when the cell divides it does so about a plane through the nucleus, and that the unit vector normal to the plane is distributed uniformly over S^2, the surface of the unit sphere in three dimensions.

Photographs were taken of cells prior to division. These photographs give a two-dimensional projection of the cell, idealised as a circle containing a point identifying the position of the nucleus. For each negative, four distances were measured; a, b, c and d represent the four distances recorded from the periphery of the cell at each of the four points of the compass to a point measured as being at the centre of the nucleus (Fig. 7). From these measurements, it is possible to calculate the radius R of the cell and also the projected distance r of the point representing the nucleus from the centre of the circle.

If a + c = x, and b + d = y

\[ R = \sqrt{\left(\frac{y}{2} - b\right)^2 + \left(\frac{x}{2}\right)^2} \]

and \[ r = \sqrt{\left(\frac{y}{2} - b\right)^2 + \left(\frac{x}{2} - a\right)^2} \]

If the vector joining the centre of the sphere to the nucleus is not in a plane perpendicular to the direction of projection, then the measure r will be less than the actual distance of the nucleus from the centre of the sphere. The radius R of the cell is assumed to be a scale factor, not influencing the proportion Q of the cell material that will go to form the smaller of the two daughter cells and so the analysis is based on the ratio r/R, called the measured eccentricity (e). If r* is the true distance of the nucleus from the centre of the sphere, then r*/R represents the real eccentricity (e*).

Two models are considered for the distribution of the unit vector defining the
direction of the line joining the centre of the sphere to the nucleus. The first model assumes that it is uniformly distributed over $S^2$ independently of the direction of projection. The second model assumes that it is orthogonal to the direction of projection, and hence that the measure $r$ is the true distance of the nucleus from the centre of the sphere rather than a projection of this distance (i.e. $e = e^*$).

In the first model it is a difficult task to reconstruct the distribution of the real eccentricity $e^*$ from observations of the measured eccentricity $e$. An integral formula can be obtained relating the distribution of $e^*$ to that of $e$ (Pogorelov, 1979, pp 54–61) but the relationship is ill-suited for inference based on sampled values of $e$. However, it is possible to estimate the distribution of $Q$ directly from the sampled values of $e$, without an intermediate stage involving estimation of the distribution of $e^*$, in the following manner. A unit vector that is uniformly distributed over $S^2$ is first selected, followed by a second unit vector in the plane orthogonal to the first, uniformly distributed over the unit circle $S^1$ in this plane. The plane in which these two unit vectors lie is then such that the unit vector normal to this plane is uniformly distributed over $S^2$, by symmetry. But now the first unit vector can be regarded as the direction of projection, and so a distribution for $Q$ can be obtained from a single observation $e$ by supposing that the observed cell divides about a plane, the unit vector normal to which is uniformly distributed over the circle $S^1$ in the plane orthogonal to the direction of projection. A calculation given in Section 2 of the Appendix shows that the distribution of $Q$ is the same as that of

$$
\frac{1}{4} \left[ 2 + e^3 \cos^3 \left( \frac{\mu}{2} \right) - 3e \cos \left( \frac{\mu}{2} \right) \right]
$$

where $\frac{\mu}{2} = \psi$, the angle between the unit vector normal to the plane of division and the line joining the nucleus to the centre of the cell, is distributed uniformly on the interval $[0, \frac{\pi}{2}]$.

In the second model, when an observation $e$ is assumed to be the real eccentricity $e^*$, the distribution of $Q$ resulting from this value of $e^*$ can be calculated directly. The angle $\psi$ will be such that $\cos \psi$ is uniformly distributed on $[0, 1]$. From this it follows that the distribution of $Q$ is the same as that of

$$
\frac{1}{4} \left[ 2 + e^3 u^3 - 3eu \right]
$$

where $u$ is uniformly distributed on the interval $[0, 1]$.

An estimator for the distribution of $Q$ can now be constructed. If $e_i$, $i = 1, 2, \ldots, n$ are the observed values of $e$ then an unbiased estimator of the probability that $q_1 \leq Q \leq q_2$ is

$$
\frac{1}{n} \sum_{i=1}^{n} \int_{0}^{1} \left[ I_{q_1 \leq 1} \left( 2 + e_i^3 f(u)^3 - 3e_i f(u) \right) \leq q_2 \right] \, du
$$
where \( q_1 \) and \( q_2 \) are chosen arbitrarily, and \( f(u) = \cos \left( \frac{\pi u}{2} \right) \) in the first model and \( f(u) = u \) in the second model. Here the function \( I \) equals one if the value of \( u \) is such that the statement in square brackets is true and equals zero otherwise.

2. **Calculations of distribution**

The derivations of the distributions (1) and (2) arising from the first and second models respectively are as follows:

The circle illustrated represents a cross-section of the cell through its centre \( C \) and the nucleus \( N \); the line \( AD \) lies in the plane of division, which is orthogonal to the line \( CB \). Under the first model \( \psi \) is uniformly distributed on \([0, \frac{\pi}{2}]\), while under the second model \( \cos \psi \) is uniformly distributed on \([0, 1]\).

To standardise units take the radius of the circle \( R \) to be unity, and let \( V_1 \) and \( V_2 \) be the volumes of the sections of the sphere lying on either side of the plane of division, with \( V_1 \leq V_2 \). The volume \( V_1 \) can be evaluated by integrating over slices parallel to the plane of division. If \( \varphi \) is the distance of such a slice from the centre \( C \) then

\[
V_1 = \pi \int_{h}^{1} (1 - \varphi^2) d\varphi
= \pi \left( \frac{2}{3} + h^3 - h \right).
\]

Now
\[
V_1 + V_2 = 4\pi
\]
\[
= \frac{3}{3}
\]
Table 5. Estimated size distribution of the smaller daughter cell in 2/16 couplets according to models 1 and 2.

<table>
<thead>
<tr>
<th>z</th>
<th>0.19</th>
<th>0.20</th>
<th>0.21</th>
<th>0.22</th>
<th>0.23</th>
<th>0.24</th>
<th>0.25</th>
<th>0.26</th>
<th>0.27</th>
<th>0.28</th>
<th>0.29</th>
<th>0.30</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 (z)</td>
<td>0.000</td>
<td>0.001</td>
<td>0.001</td>
<td>0.002</td>
<td>0.002</td>
<td>0.003</td>
<td>0.005</td>
<td>0.006</td>
<td>0.007</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2 (z)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.002</td>
<td>0.002</td>
<td>0.003</td>
<td>0.004</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>z</th>
<th>0.31</th>
<th>0.32</th>
<th>0.33</th>
<th>0.34</th>
<th>0.35</th>
<th>0.36</th>
<th>0.37</th>
<th>0.38</th>
<th>0.39</th>
<th>0.40</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 (z)</td>
<td>0.008</td>
<td>0.013</td>
<td>0.008</td>
<td>0.012</td>
<td>0.020</td>
<td>0.021</td>
<td>0.027</td>
<td>0.035</td>
<td>0.039</td>
<td>0.036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2 (z)</td>
<td>0.006</td>
<td>0.007</td>
<td>0.007</td>
<td>0.008</td>
<td>0.011</td>
<td>0.014</td>
<td>0.017</td>
<td>0.022</td>
<td>0.028</td>
<td>0.030</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>z</th>
<th>0.41</th>
<th>0.42</th>
<th>0.43</th>
<th>0.44</th>
<th>0.45</th>
<th>0.46</th>
<th>0.47</th>
<th>0.48</th>
<th>0.49</th>
<th>0.50</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 (z)</td>
<td>0.038</td>
<td>0.045</td>
<td>0.062</td>
<td>0.075</td>
<td>0.070</td>
<td>0.087</td>
<td>0.089</td>
<td>0.091</td>
<td>0.085</td>
<td>0.094</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2 (z)</td>
<td>0.035</td>
<td>0.039</td>
<td>0.050</td>
<td>0.063</td>
<td>0.073</td>
<td>0.087</td>
<td>0.101</td>
<td>0.115</td>
<td>0.122</td>
<td>0.138</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

and so

\[ Q = \frac{V_1}{V_1 + V_2} = \frac{1}{4} [2 + h^3 - 3h]. \]

But

\[ h = r \cos \psi, \]

leading to the distributions (1) and (2) respectively under the two possibilities for the distribution of the angle \( \psi \).

3. Statistical discussion

Table 5 and Figure 9 summarise the estimated distribution of \( Q \), obtained by numerical integration from formula (3). Lines 2 and 3 of Table 5 give the estimated probabilities based on models 1 and 2 respectively, with \( P_1 (z) \) or \( P_2 (z) \) being the estimated probability that \( Q \) lies in the interval \( (z - 0.01, z) \). These estimates are based on \( n = 140 \) observations \( e_i \).

The second data set consists of measurements on pairs of daughter cells, each pair giving four numbers \( x, y, g \) and \( h \) (Fig. 8). The daughter cells are idealised as ellipsoids with the smaller ellipsoid having axes \( x, x \) and \( g \), and the larger ellipsoid having axes \( y, y \) and \( h \). A measurement of \( Q \) is then given by

\[ \frac{x^2g}{x^2g + y^2h} \]

A total of \( m = 128 \) such observations was available and these are summarised in Table 6 and Figure 10. In line 2 of Table 6 the quantity \( Q(z) \) is the number of
Table 6. Comparison of estimated and measured size distributions of the smaller daughter cell in 2/16 couplets.

<table>
<thead>
<tr>
<th>$z$</th>
<th>0.31</th>
<th>0.32</th>
<th>0.33</th>
<th>0.34</th>
<th>0.35</th>
<th>0.36</th>
<th>0.37</th>
<th>0.38</th>
<th>0.39</th>
<th>0.40</th>
<th>0.41</th>
<th>0.42</th>
<th>0.43</th>
<th>0.44</th>
<th>0.45</th>
<th>0.46</th>
<th>0.47</th>
<th>0.48</th>
<th>0.49</th>
<th>0.50</th>
<th>Totals*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q(z)$</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>8</td>
<td>5</td>
<td>12</td>
<td>9</td>
<td>15</td>
<td>7</td>
<td>10</td>
<td>9</td>
<td>13</td>
<td>11</td>
<td>7</td>
<td>128</td>
</tr>
<tr>
<td>$mP_1(z)$</td>
<td>1.6</td>
<td>1.0</td>
<td>1.6</td>
<td>2.5</td>
<td>2.6</td>
<td>3.4</td>
<td>4.4</td>
<td>4.9</td>
<td>4.6</td>
<td>4.8</td>
<td>5.7</td>
<td>7.9</td>
<td>9.6</td>
<td>9.0</td>
<td>11.1</td>
<td>11.4</td>
<td>11.6</td>
<td>10.9</td>
<td>12.0</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Discrepancy</td>
<td>2.5</td>
<td>1.1</td>
<td>3.1</td>
<td>4.0</td>
<td>0.2</td>
<td>3.0</td>
<td>0.4</td>
<td>0.1</td>
<td>0.5</td>
<td>0.2</td>
<td>0.0</td>
<td>2.1</td>
<td>17.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$mP_2(z)$</td>
<td>0.7</td>
<td>0.9</td>
<td>0.9</td>
<td>1.0</td>
<td>1.5</td>
<td>1.8</td>
<td>2.2</td>
<td>2.8</td>
<td>3.5</td>
<td>3.9</td>
<td>4.5</td>
<td>5.0</td>
<td>6.4</td>
<td>8.1</td>
<td>9.4</td>
<td>11.1</td>
<td>13.0</td>
<td>14.8</td>
<td>15.7</td>
<td>17.6</td>
<td>128</td>
</tr>
<tr>
<td>Discrepancy</td>
<td>0.0</td>
<td>0.0</td>
<td>7.7</td>
<td>5.9</td>
<td>1.0</td>
<td>6.0</td>
<td>0.6</td>
<td>0.1</td>
<td>1.2</td>
<td>0.2</td>
<td>1.4</td>
<td>6.4</td>
<td>30.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The individual values are each rounded off to the nearest decimal point, and therefore their sum does not always equal the total value.
observations in the interval \((z - 0.01, z)\). In lines 3 and 5 the expected numbers of such observations under models 1 and 2 respectively are recorded. Lines 3 and 5 contain discrepancies of the form

\[
(4) \quad \frac{(Q(z) - m P_j(z))^2}{m P_j(z)} \quad z \geq 0.43
\]

for \(j = 1, 2\), that is for models 1 and 2, respectively. To calculate discrepancies for \(z > 0.43\) the ranges \(0 \leq z \leq 0.35, \ 0.35 < z \leq 0.38, \ 0.38 < z \leq 0.40\) and \(0.40 < z \leq 0.42\) were formed into single intervals so as to ensure that no entries substituted into expression (4) fell below 6. If the observations \(Q(z)\) came from the distributions \(P_j(z)\) then the total discrepancy over the 12 intervals would have approximately a chi-squared distribution with 11 degrees of freedom. The total discrepancy is 17.3 for model 1 and 30.6 for model 2, while \(\chi^2_{11}(90) = 17.3, \ \chi^2_{11}(95) = 19.7, \ \text{and} \ \chi^2_{11}(99.5) = 26.8\). Since the quantities \(P_j(z)\) are estimates of the distribution underlying the observations \(Q(z)\) we should expect the total discrepancies to be somewhat larger than is indicated by a \(\chi^2_{11}\) distribution. It is difficult to assess this effect precisely\(^1\), but it is unlikely that it would be great enough to prevent the hypothesis of interest being rejected under model 2. Under model 1 the hypothesis of interest is found to be consistent with the two data sets.

\(^1\)To put an upper bound on this effect chi-squared tests were performed on the \(2 \times 12\) contingency tables formed from lines 2 and 3 or lines 2 and 5 of Table 6. The (nominally \(\chi^2_{11}\)) statistic obtained was 8.6 for model 1 and 13.9 for model 2. The smoothing involved in formula (3) suggests that the actual effect is much smaller than this.