MOVEMENT OF THE CELL SURFACE AND CHANGE IN SURFACE AREA DURING CLEAVAGE IN THE NEWT'S EGG

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SUMMARY
In cleaving eggs of the newt, *Cynops pyrrhogaster*, the behaviour of the egg surface was analysed by observing linear and area changes in a transplanted piece of cortex which differed in pigmentation from the background.

Results from the animal hemisphere showed that the surface in the cleavage plane shrank extensively in the region where the early cleavage furrow was being formed. On both sides of the cleavage plane, the surface began to shrink in a direction parallel to the cleavage furrow and to stretch perpendicularly to the furrow, simultaneously with the onset of cleavage. The nearer the grafted region was to the furrow, the greater the change. These changes continued until about the time of the appearance of unpigmented surface on both walls of the furrow, when a maximum was reached. Thereafter a slight reversal was observed. Similar changes of lesser magnitude were encountered in regions remote from the cleavage plane. The surface area decreased in the vicinity of the furrow during the early phase of cleavage and returned roughly to its original value during the late phase, while, in the distant region, only an increase occurred.

Results from the vegetal hemisphere showed that little linear change of surface occurred even in the region adjacent to the furrow, while the surface area changed in the furrow region similarly to the animal half. In the vegetal hemisphere remote from the furrow, the changes took place to a lesser extent.

INTRODUCTION
The behaviour of the egg surface has been studied in detail during cleavage of the echinoderm egg. Concerning linear changes in the surface, in the region close to the cleavage furrow, the surface shrank along the circumference of the cell at the initial stage of the cleavage, after which it stretched gradually as cleavage advanced. Only elongation was observed in the region far from the furrow (Dan, Yanagita & Sugiyama, 1937; Dan, Dan & Yanagita, 1938; Dan & Dan, 1940; Hiramoto, 1958). Concerning changes in surface area during cleavage, a decrease was followed by an increase in the region near the furrow, while only an expansion occurred in the distant part (Dan & Ono, 1954; Hiramoto, 1958).

In the animal half of the amphibian egg during cleavage, linear contraction of the surface occurred in a direction parallel to the cleavage furrow and stretching occurred perpendicular to the furrow, while the surface area changed slightly (Selman & Waddington, 1955).

Selman & Waddington (1955) and Zotin (1964) explained the entire process of cytokinesis in the amphibian egg in such a way that, in the initial phase of cleavage, the
furrow was formed by contraction of the cortex along the cleavage plane, and in the late phase, new surface was formed along the diastema which split the egg in 2 halves. Recently, electron-microscopic observations suggested that the contraction of the cortex was brought about by a band of microfilaments underlying the bottom of the furrow (Bluemink, 1970, 1971; Selman & Perry, 1970; Kalt, 1971) and the new surface was supplied by coalescence of membrane-bound vesicles with the existing furrow surface (Bluemink, 1971; Bluemink & de Laat, 1973; Kalt, 1971).

It seemed opportune to examine in detail the surface movement in the light of the leading hypotheses of amphibian cytokinesis. For this purpose, linear and area changes in the surface were locally investigated in the newt egg using the technique of transplanting cortex distinguishable from that of the host by virtue of a difference in the degree of pigmentation.

MATERIALS AND METHODS

Naked eggs of the newt, Cynops (Triturus) pyrrhogaster, were obtained by procedures described previously (Sawai, 1972). The eggs were operated and observed in Holtfreter's solution with double CaCl₂ concentration. It seemed that the additional Ca-ions accelerated recovery from the incision (cf. Gingell, 1970). The eggs were put on agar gel in a shallow depression of such a size that, after equilibrium, an egg became about 3 mm across. Operations were performed under a dissection binocular microscope, free-hand, at room temperature (20-25 °C).

Transplantation of the cortex was made with a fine glass needle (Sawai, 1974). From the position in the recipient egg where transplantation was to be made, a piece of cortex was removed. Then, cortex was cut from another egg and endoplasm lining the piece was removed as much as possible. The remaining thin cortex was grafted at the selected site on the recipient. The recipient was always an uncleaved fertilized egg. The graft was derived from the animal half of an egg before the 4-cell stage and its final size after transplantation was 0.1-0.3 mm square. After the transplant was fully stabilized on the host egg (15-30 min after the transplantation), photographs were taken every 5 min for as long as the contour of the graft could be traced. In many cases the photography was begun before the start of the cleavage. Measurements were performed directly on prints enlarged to the same degree.

Linear changes of the surface were measured with transparent section paper ruled into 1-mm squares. By placing one axis of the ruled paper parallel to the cleavage plane, the surface movements in 2 directions in one region of an egg could be measured at the same time.

Area changes in the surface were measured using a transparent plate with small black dots 2 mm apart. The plate was put on the picture and number of the dots located within the contour of the graft + half the number of dots on the perimeter of the contour were counted. The counting was repeated 3 times on each photograph to obtain a mean value and the direction of the plate was changed each time.

All values for the linear and the area measurements were indicated as percentages of the initial values.

Under the present experimental conditions, the curvature of the surface changed locally a little especially in the animal half of the egg during cleavage (Fig. 1). Therefore, a slight error was unavoidable when measurements were made on the photograph. By estimation from the animal half in Fig. 1, linear measurements on the print were underestimated by 2-7% in comparison with the true length during cleavage. A change in curvature in the vegetal half was restricted to the region adjacent to the furrow. Whether on the animal or on the vegetal half, measurements on the periphery were excluded because the transplants tended to be shifted toward the edge of that part of the egg which could be seen on the photograph, causing large errors.
Results

Linear change in the surface during the cleavage

When furrow formation started on the graft, a marked constriction appeared within the furrow plane of the graft (Fig. 2). Shrinkage along the furrow reached a maximum of about 80% decrease from the initial value at about 20 min after the start of the cleavage (Fig. 4A) and the graft took a dumbbell shape (Fig. 2E). Subsequently, the surface in the furrow region of the graft was replaced by unpigmented surface appearing on both sides of the furrow (Fig. 2F). A graft placed ahead of the advancing tip of the host furrow was first pulled toward the furrow tip (Fig. 3B, C) and when the furrow was traversing the graft, the latter contracted in the same fashion as in Fig. 2 (Figs. 3D–F, Fig. 4B. See also Sawai, 1974). The present author reported in an earlier paper that progress of the furrow was temporarily arrested when the furrow tip met the graft (Sawai, 1974). However, no such temporary arrest was observed in the present experiment, probably because the graft was stabilized on the host egg.

Grafts in the path of the furrow become separated into 2 parts during cleavage. This situation is fortunate because it enables one to follow linear changes in pieces which are too small to be transplanted independently. Consequently, at the first moment when the position of the future cleavage furrow became detectable and when most linear changes had not yet started, widths of the transplant extending out beyond the furrow plane on both sides were measured. By these measurements, the separating parts were classified into 3 groups by size (w), w ≤ 0.2; 0.2 < w ≤ 0.3; and 0.3 < w ≤ 0.5 mm. Diameters of the half pieces were determined in directions parallel to and perpendicular to the furrow plane. Results in each region are shown in Fig. 5A–C. The graft was stretched perpendicularly to and contracted parallel to the furrow (see again
Fig. 2A–F. Change in shape at 5-min intervals of a graft placed on animal pole during the cleavage. f, furrow; g, graft. ×14.

Fig. 3A–F. Shape changes at 5-min intervals of graft placed ahead of furrow tip during cleavage. f, furrow; g, graft. ×14.
Behaviour of newt egg surface during cleavage

These changes also reached a maximum at about the time when contraction along the furrow attained a maximum, and thereafter there was a tendency to return toward the original levels. Extreme values for the extension were about 240, 120 and 30% increase, respectively, for the 3 groups, while the shrinkage was about 60, 40 and 30% decrease (Fig. 6). The charges were greater in the region nearer the furrow.

The following observations and measurements were made on grafts transplanted to parts of the animal half at various distances from the cleavage plane. Figs. 7–11 show features of the deformation of the transplants. In a position adjacent to the initial furrow, the graft elongated toward the furrow and shrank in the direction parallel to the furrow (Fig. 7). The graft at a short distance from the furrow also behaved similarly (Fig. 8). When transplantation was made ahead of the furrow tip but in a position adjacent to its future path, the graft was pulled toward the advancing tip of the furrow, becoming curved, as the tip touched the graft (Fig. 9). In a region far from the furrow plane, the graft stretched parallel to and shrank perpendicularly to the furrow, i.e. the direction of stretching and shrinkage on the photograph (Fig. 10) was opposite to that in a region near the furrow.

In Fig. 10, the graft shifted toward the periphery as the furrow advanced. The shifting must be caused by both the stretching of the surface intervening between the graft and the furrow and also by growth of the unpigmented surface. The former is obvious from the results described previously (Fig. 6). The latter was confirmed by the observation that, when intervening pigmented surface elongated little, the graft shifted with
growth of the pale surface. The degree of shifting was proportional to the extent of the pale surface (Figs. 10, 11).

The direction of the extension corresponded roughly in almost all regions examined to the movement of pigment granules observed by Selman & Waddington (1955).

Fig. 5. Graphical representation of the linear changes on the 2 sides of the furrow plane; 3 representative cases in the small (A), medium (B) and large (C) half pieces. Measurements in the direction parallel (o) and perpendicular (●) to the furrow for each half piece are connected by the same kind of line.

Fig. 6. Average values of available data in small (—), medium (—●—), and large (———) groups of half pieces.

The magnitude of the linear changes across each graft was determined according to its distance from the cleavage plane; 0.03–0.3, 0.2–0.7, 0.4–0.9 and 0.7–1.2 mm. These categories overlapped unavoidably with one another because of a technical difficulty of transplanting smaller grafts of the same size. The first 3 regions correspond roughly to the positions of the grafts in Figs. 7, 8 and 10, respectively. Fig. 12A–D shows results in each region. The curves of the first group began to change at about the time of the
Fig. 7A–D. Changes in shape at 5-min intervals of graft adjacent to furrow during cleavage. $f$, furrow; $g$, graft. $\times 14$.

Fig. 8A–D. Shape changes at 10-min intervals of graft transplanted a short distance from furrow during cleavage. $f$, furrow; $g$, graft. $\times 14$.

Fig. 9A–D. Shape changes at 10-min intervals of graft adjacent to second cleavage plane during second cleavage. $g$, graft; $f$ and $sf$, first and second cleavage furrows, respectively. $\times 15$. 
Figs. 10, 11A–D. Changes in shape at 10-min intervals of grafts transplanted on animal half far from cleavage plane. f, furrow; g, graft. × 15.
start of the cleavage, reaching extreme values of about 35% elongation and 40% shrinkage 15-20 min later (Fig. 12A). The values returned slightly toward the original level in the late phase of the cleavage. A similar behaviour was found in the second group, but to a lesser degree and with a delay (Fig. 12B). In curves of the 2 remaining groups, the directions of the changes were reversed and a return from extreme values was not seen (Fig. 12C, D). The relationship between the time after the start of cleavage and the appearance of the initial sign of the shape change in the animal half was examined on the grafts placed to the animal pole (where all cleavage started) and to 3 positions classified by their nearest distance \(d, \text{mm}\) from the animal pole, \(0 < d \leq 0.1; 0.1 < d \leq 0.3;\) and \(0.3 < d\) (Table 1).
Table 1. Relationship between the position of grafts on the animal half and the time after the start of cleavage at which shape changes began

<table>
<thead>
<tr>
<th>Time after start of cleavage, min</th>
<th>The nearest distance (d, mm) to the graft and number of cases in each region</th>
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<tbody>
<tr>
<td>0</td>
<td>0 &lt; d ≤ 0.1 0.1 &lt; d ≤ 0.3 0.3 &lt; d</td>
</tr>
<tr>
<td>0</td>
<td>9 6 1</td>
</tr>
<tr>
<td>5</td>
<td>1 2 7 1</td>
</tr>
<tr>
<td>10</td>
<td>1 3 2</td>
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<tr>
<td>15</td>
<td>-- -- 1</td>
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<td>20</td>
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* Just after start of the cleavage.
† On animal pole

Fig. 13. Graphical representation of the linear changes in the vegetal half, measured perpendicularly to the furrow in 3 regions; 0-0-3 (---), 0-1-0-5 (---) and 0-3-0-6 (---) mm distant from the furrow plane. Time 0 is taken as the time when the tip of the host furrow reached the same level as the graft.

The results from the vegetal half are shown in Figs. 13–15. The surface in the cleavage plane was pulled toward the furrow tip in the same way as was seen in the animal half (Fig. 14A, B). However, an obvious contraction of the cortex, as in the animal half, did not occur. The graft was separated into two parts by the furrow, as if cut off with a knife (Fig. 14C, D), making it impossible to measure the magnitude of the contraction along the furrow. In any region except the furrow, the shape of the graft changed only slightly even in a position adjacent to the cleavage plane (Figs. 13, 15).

Change in the surface area during cleavage

Measurements of surface area on the animal half were performed on grafts transplanted to 4 positions classified by their distance from the cleavage plane; 0 (on the cleavage plane), 0.03–0.3, 0.2–0.9 and 0.7–1.2 mm. In the first region, since the graft was eventually separated into 2 pieces by the unpigmented surface, the areas of the 2 pieces were summed. Fig. 16A–D shows the results in each region. In the transplant on the cleavage plane, a gradual decrease in area continued from the onset of cleavage until about the time of the appearance of unpigmented surface, reaching a minimum
Fig. 14A–D. Shape changes at 10-min intervals of graft placed on cleavage plane of vegetal half. ×15.

Fig. 15A–D. Shape changes at 10-min intervals of graft adjacent to cleavage plane of vegetal half. ×15.
of about 30% decrease (Fig. 16A). Thereafter, the area expanded, returning roughly to the original value. In the second region, the area changed with practically the same pattern as the first region (Fig. 16B). Little change in area was found in the third group (Fig. 16C). The surface area of the last group increased gradually with the advance of the cleavage, reaching a maximum of about 20% increase during observation (Fig. 16D).

Fig. 16. Changes in surface area in the animal half during cleavage. In transplants in 4 ranges: A, the cleavage plane; and B, 0-03-0-3; C, 0-2-0-9; and D, 0-7-1-2 mm distant from the cleavage plane. Values show % change in surface area from the initial value (100%). Abscissa: time −, 0 and + are before, just at and after the onset of the cleavage, respectively.

In the vegetal half, the area of a graft on the furrow showed a similar pattern of change to that in the animal half, when the furrow was travelling on the vegetal surface (Fig. 17A), but there was little change in the regions distant from the furrow (Fig. 17B).
**DISCUSSION**

The surface movement of the newt egg differed in some respects from the echinoderm egg. Concerning linear changes, in the echinoderm the surface close to the furrow shrank along the circumference at the initial stage of the cleavage (Dan et al. 1937, 1938; Dan & Dan, 1940; Hiramoto, 1958), but not in the newt. In the echinoderm there was no difference in magnitude of the change between the animal and vegetal halves (Dan et al. 1938), while in the newt egg there was a big difference between the 2 halves. Regarding changes in area, although the pre-existing surface expanded in all regions of echinoderm egg in the latter half of the cleavage process (Dan & Ono, 1954; Hiramoto, 1958), expansion in the newt eggs occurred only in positions far away from the furrow plane.

Both the linear and the area changes in the *Cynops pyrrhogaster* egg were larger than those in the *Triturus alpestris* egg (Selman & Waddington, 1955). In *Xenopus laevis* egg cleaving in a medium with cytochalasin B, in which the cleavage furrow was retracted but unpigmented surface was exposed, the pre-existing pigmented surface never shrank nor stretched (Bluemink & de Laat, 1973). In *C. pyrrhogaster* egg in Holtfreter solution, a little movement of the pigmented surface was observed even after the appearance of unpigmented surface.

In connexion with current theory of amphibian cytokinesis (Bluemink, 1970, 1971; Bluemink & de Laat, 1973; Kalt, 1971; Selman & Perry, 1970; Selman & Waddington, 1955; Zotin, 1964), the extreme shrinkage of the surface along the furrow plane may directly reflect the furrow formation owing to the contraction of the cortex. The linear and the area changes in the vicinity of the furrow attained extreme values when the pale surface appeared on both sides of the furrow. These observations suggest that only the early process of furrow formation is brought about by contraction, and they also
support the idea that unpigmented surface grows as an addition of new membrane to the existing furrow surface.

In the vegetal half, slight shrinkage observed on the surface along the furrow may indicate that contraction of the cortex is effective in making a shallow furrow, but not in the deeper layers. That the surface movement is so small in the vegetal half may be due to the high viscosity of the cortical cytoplasm of this half (Selman & Waddington, 1955; Sawai & Yoneda, 1974).

The present author reported in a previous paper that subcortical cytoplasm underlying the furrow could induce a furrow on whichever part of the surface below which the cytoplasm was transplanted. The effective cytoplasmic component was always localized along the leading edge of the furrow from initiation of cleavage to the late phase (Sawai, 1972). By electron-microscopy, the microfilament structure was found not only beneath the bottom of the initial furrow, but also along the advancing end of the furrow for some time after the appearance of the pale surface (Selman & Perry, 1970). These observations suggest that we cannot exclude the possibility that contraction of the cortex participates in furrow formation after the appearance of the pale surface.

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REFERENCES


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