ROLES OF CORTICAL AND SUBCORTICAL COMPONENTS IN CLEAVAGE FURROW FORMATION IN AMPHIBIA

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SUMMARY

In the eggs of the newt, *Triturus pyrrhogaster*, 2 separate factors are recognized which take part in cleavage furrow formation. (1) The inductive capacity for the furrow formation by the cytoplasm lying under the cortex along the cleavage furrow (FIC); and (2) the reactivity of the overlying cortex to form a furrow in response to FIC.

(1) *FIC.* The inductive capacity is shown by the fact that FIC induces a furrow on whichever part of the surface under which FIC is transplanted. FIC is distributed along the cleavage furrow and even extends along the future furrow plane ahead of the furrow tip. The distance FIC precedes the furrow tip is about 1·0 mm in the animal hemisphere and is less in the vegetal hemisphere. In the direction at right angles to the furrow plane, FIC does not spread more than 0·1 mm.

FIC is also present in the eggs of *Xenopus laevis*. Species specificity of FIC for induction is not found between *Triturus* and *Xenopus*.

(2) *Surface layer.* At the onset of the first cleavage, the reactivity of the cortex to form the furrow in answer to FIC induction is localized on the animal pole region. The reactivity of the cortex propagates medially as a belt along the surface towards the vegetal pole with the advancing tip of the cleavage furrow. After the furrow is completed, the reactivity begins to be lost from the animal pole region, and eventually over the entire surface. The reactivity, however, reappears on the animal pole region simultaneously with the second cleavage.

INTRODUCTION

Various hypotheses or interpretations have been brought forward with regard to the mechanism of cytokinesis in animal cells.

Marsland & Landau (1954) proposed a 'contractile ring theory'. Recently, the theory found support in electron-microscopic observations in that filamentous structures were recognized on the bottom of the deepening cleavage furrow in several forms (*Stomatoca*, Schroeder, 1968; *Loligo*, Arnold, 1969; *Arbacia*, Tilney & Marsland, 1969; *Aequorea*, Szollosi, 1970; *Triturus*, Selman & Perry, 1970; *Ambystoma*, Bluemink, 1970).

Rappaport (1969) considered that the active contraction of the surface of the division plane was brought about on the reaction site of 2 asters.

Kinoshita and others (Kinoshita & Hoffmann-Berling, 1964; Kinoshita & Yazaki, 1967; Kinoshita, 1968) understood the mechanism of the cell division in such a way that the cortex which was in a contractile state over the entire surface before cleavage was locally relaxed at the region surrounding the spindle poles by the effect of the

relaxing factor, so that the cortex of the division plane which retained a higher tension was automatically constricted until the cell was divided.

Although the above authors regard the superficial structures as playing a leading part in cytokinesis, there are reports to the contrary, emphasizing the fusion of vesicles resulting in the formation of a new cell membrane, as in plant cells (Motomura, 1960; Buck & Tisdale, 1962; Humphreys, 1964; Murray, Murray & Pizzo, 1965; Thomas, 1968).

Concerning amphibian cleavage, Selman & Waddington (1955) and Zotin (1964) reported that subsequent to the 'dipping in' of the surface by the contraction of the cortex of the cleavage plane, a septum is formed along the diastema.

In all the above-mentioned studies, in spite of the fact that local changes accompanying division were discussed in theory, the materials actually dealt with were whole eggs. Therefore, in order to make any further advance in the understanding of cytokinesis, it is necessary to analyse local changes in dividing cells; this has been done in work reported in the present paper, using the amphibian egg.

Among Japanese workers, Dan & Kojima (1963) showed that, in newt eggs, some change in the cortex which is indispensable for furrow formation leads the advance of the cleavage furrow. For instance, a furrow could be completed on a piece of isolated cortex as long as it included an area ahead of a furrow tip.

In frog eggs, Kubota (1969) suggested the existence of special subcortical cytoplasm necessary for the induction of a cleavage furrow. The suggestion was based on observation that dislocation of the subcortical cytoplasm lying beyond the furrow tip, by rubbing the egg with a hair loop, bent the path of the furrow which appeared later. Sawai, Kubota & Kojima (1969) confirmed Kubota's observations in the newt eggs.

In the present paper, detailed analyses were made with regard to the separate roles of the subcortical and cortical components in forming the cleavage furrow by transplantation of subcortical cytoplasm under the cortex at different locations.

MATERIALS AND METHODS

The eggs of *Triturus pyrrhogaster* and *Xenopus laevis* were used. Spawning of the newt eggs was stimulated by grafting cattle hypophyses which were lyophilized and cut in small pieces (about 2 mm^3); in the case of the frog, by injecting 250 i.u. of pituitary hormone (Gonatropine, Teikoku Zoki Co. Ltd) into both females and males.

The jelly coat and the vitelline membrane of the newt eggs were manually removed with scissors and watchmaker's forceps. The jelly coat and the vitelline membrane of the frog eggs were removed with steel needles and forceps. The naked eggs were operated in Holtfreter's solution in a depression of agar gel covering the bottom of the dish.

Transplantation of the cytoplasm was achieved with glass tubing, one end of which had been drawn into a capillary about 50 μm in diameter, and the other connected to rubber tubing to be sucked by mouth. After insertion, the capillary was pushed through the egg and brought close to the egg surface of the opposite side so that the surface was slightly pushed out by the capillary tip. Hiramoto performed a similar experiment in sea-urchin egg (1957) and showed that when the egg surface began to be raised the capillary tip had reached the cortical gel layer. It was known from previous investigation (Sawai *et al.* 1969) that when Nile blue was injected at this site in the egg the colour of the dye could be discerned from the outside and that the coloured patch was dislocated when the egg surface was rubbed with a hair loop. The newt cytoplasm sucked into the capillary from such a subcortical site will be called the sub-cortical cytoplasm in the present paper.

For transplantation within the same egg, the capillary was turned to a new position which was again indicated by a small bulge on the surface, and the subcortical cytoplasm in the capillary was blown out there. After transplantation the region was recognizable by a slight difference in shade observable from the outside of the egg; since the capillary was pushed through the cell to the opposite side, no scar was made over the point of transplantation. But in the majority of cases, the sucked subcortical cytoplasm was deposited in another egg in the same way. The volume of transplanted subcortical cytoplasm was about 10^{-4} to 10^{-5} ml. All operations were performed under the dissecting binocular microscope, free-hand, at room temperature (18–25 °C).

RESULTS

Furrow-inducing capacity of subcortical cytoplasm

Induction of a cleavage furrow in lateral surface by the subcortical cytoplasm taken from under the furrow tip. Sawai et al. (1969) reported that transplanted subcortical cytoplasm taken from under the advancing tip of the cleavage furrow induced a dent when placed under the lateral surface around the spindle pole. More detailed information will be given here.

When the first cleavage furrow of *Triturus* egg advanced one third of the egg diameter from the animal pole, the subcortical cytoplasm was taken from under the furrow tip and it was transplanted beneath the cortex of the lateral region midway between the animal and vegetal poles (equator) (Fig. 1, circle). In 25 cases out of 50, the furrow-like dents were formed there (Table 1, Fig. 9 B). The reaction was positive regardless of whether the donor and the recipient were the same egg or different ones.
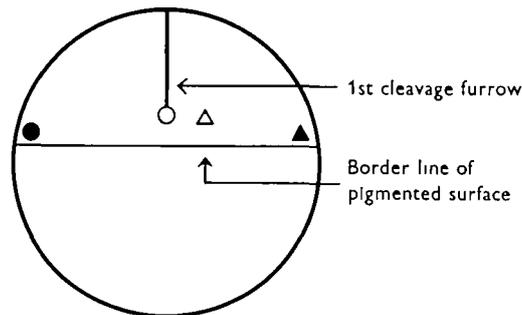

Fig. 1. Positions of removal (○, △) and deposition (●, ▲)
of the subcortical cytoplasm.

Table 1. *Success and failure in dent formation by the subcortical cytoplasm of the furrow tip and beside the tip*

Site of cytoplasm taken	No. of transplants	Results		
		+	±	−
Furrow tip	50	25	1	24
Side of furrow tip	50	0	0	50

+, positive; ±, weak; −, negative.

As a control, the subcortical cytoplasm of a region a short distance to the side of the furrow tip was transplanted (Fig. 1, hollow triangle). No dent was formed (Table 1).

Next, samples of furrow tip subcortical cytoplasm derived from 4 stages (A, B, C, and D) defined by the progress of the first cleavage furrow (Fig. 2) were tested for capacity to induce dents. Recipients were always at stage A of Fig. 2. The furrow-tip cytoplasm of each stage induced a dent on the lateral equatorial surface of recipient eggs (Table 2). In the Table the percentages of positive cases at stages A and B did not show a significant difference, while the percentages decreased as the stage proceeded from C to D. Decrease in the inductive capacity toward the vegetal pole may be due to a difference in the quantity of yolk granules involved in the transplant. The dent was also induced by furrow-tip cytoplasm of the second cleavage stage. Control experiments, placing subcortical cytoplasm derived from beside the furrow tip, were all negative.

In contrast to transplantation, when a large amount of furrow-tip cytoplasm was removed by sucking, the furrow was not formed in this deprived region.

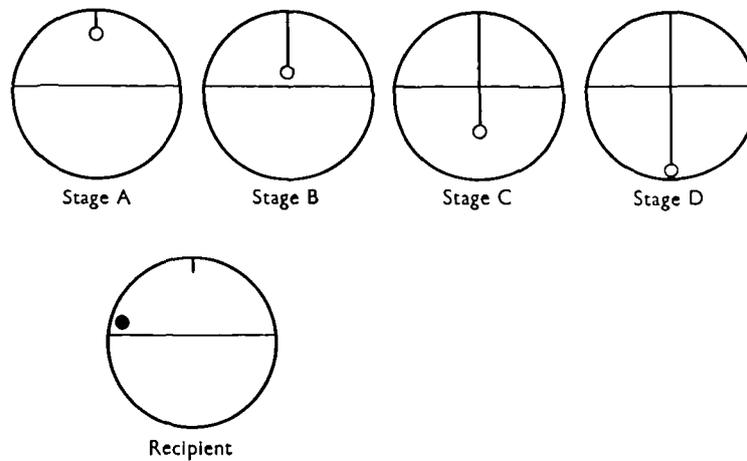Various events accompanying dent formation are as follows. (1) Pigment granules

Fig. 2. Stages of donor egg for testing the inductive capacity of the furrow tip cytoplasm (open circle). Stage of recipient egg and position of transplantation (solid circle).

Table 2. *Dent induction by the subcortical cytoplasm of the furrow tip of 4 cleavage stages*

Stage of donor	No. of transplants	Results		
		+	±	−
A	10	7	1	2
B	10	8	1	1
C	14	8	0	6
D	16	5	1	10

Symbols as in Table 1.

Table 3. *Exchange transplantation of the furrow tip cytoplasm between newt and frog eggs*

Donor	Recipient	No. of cases	Results +	Results ±	Results −
Newt	Frog	7	5	0	2
Frog	Newt	2	2	0	0

Symbols as in Table 1.

often accumulated in the transplanted region prior to dent formation (Fig. 9A), but dent formation did not necessarily follow the accumulation of pigment granules. (2) In paraffin sections, the pigment layer of the cortex was found to be considerably thicker at the dented area (also see Gingell, 1970). (3) The process of dent formation is initiated by local wrinkling (Fig. 9A). (4) Dent formation is limited to the transplanted area (Fig. 9B), the size and the form of the dent depending largely on those of the transplanted cytoplasm, for example, linear, circular or ring-shaped. (5) When a dimple-like depression is induced by placing the subcortical cytoplasm as a single mass, a pale surface with fewer pigment granules characteristic of the natural furrow is rarely formed. But when a large amount of the furrow tip cytoplasm is placed in a line, such a pale surface is quite evident (Fig. 9C). (6) After transplantation, it takes 5–30 min before a dent is recognized. (7) Dents are eventually smoothed over various time periods, from 20–30 min to more than 3 h.

The furrow-tip cytoplasm of *Xenopus* eggs also has an inductive capacity over its own cortex. Furthermore, in exchange transplantation experiments between *Xenopus* and *Triturus* eggs, the furrow-tip cytoplasm of either species induced a dent in the other (Table 3).

Hereafter, the subcortical cytoplasmic component which induces the cleavage furrow on the surface will be called the furrow-inducing cytoplasmic component (FIC).

Distribution of the furrow-inducing cytoplasmic component (FIC) around the furrow tip. This section describes experiments to determine (1) whether or not the FIC is present along the entire arc of leading edge of the in-cutting furrow, (2) how far it extends beyond the advancing tip of the cleavage furrow, and finally (3) how far to the side of the furrow it spreads.

For testing the first question, the donor eggs were classified into 4 stages as shown in Fig. 3. All the recipient eggs were at a stage when the furrow traversed one third of the egg diameter and transplantation was made on the lateral equatorial region. Cytoplasm taken from the bottom of the arc of the furrow of all 4 stages tested induced the cleavage furrow (Table 4). Reciprocally, when a large amount of subcortical cytoplasm was removed from the bottom of a furrow, the cleavage furrow was eliminated at the area of removal.

For the second question, the presence of FIC was mapped along the extrapolated
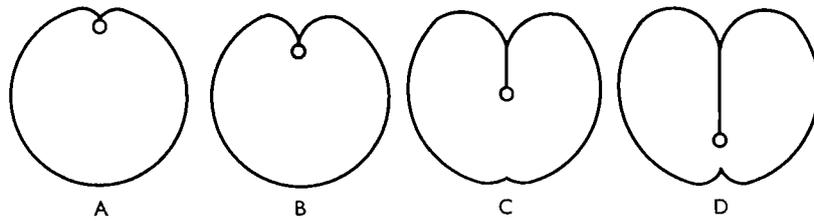
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Fig. 3. Stages of donor egg for testing the inductive capacity of the cytoplasm
lining the bottom of the arc-shaped furrow (open circles).

Table 4. *Inductive capacity of FIC derived from the furrow bottom*
of 4 stages

		Results		
Stage of donor	No. of cases	+	±	−
A	11	4	2	5
B	14	5	0	9
C	20	8	2	10
D	17	8	2	7

Symbols as in Table 1.

Table 5. *The distribution of FIC ahead of the furrow tip*

		Results		
Stage and distance from furrow tip, mm	No. of cases	+	±	−
A: 1·0	14	4	3	7
B: 1·0	13	1	1	11
C: 0·3	15	0	3	12
D: 0·1	17	6	2	9
E: 0	11	7	0	4

Symbols as in Table 1.

line of the furrow beyond the tip. The stage of the donor used for the animal hemisphere
was just after the first cleavage furrow appeared on the animal pole region. The
recipient egg was at the same stage, transplantation being made at a lateral location
(Fig. 4A). The results indicated that FIC preceded the visible tip of the furrow all
across the pigment cap (1 mm); 1·0 mm was the farthest distance from the visible
furrow tip giving positive results (Table 5, A).

For the vegetal hemisphere, since it is rather cumbersome to map out the distribu-
tion of FIC for different stages, the technique was simplified. A point was fixed from
which the subcortical cytoplasm was to be taken, and the time of removal of the
subcortical cytoplasm (stage) so selected that the furrow tip was away from the point
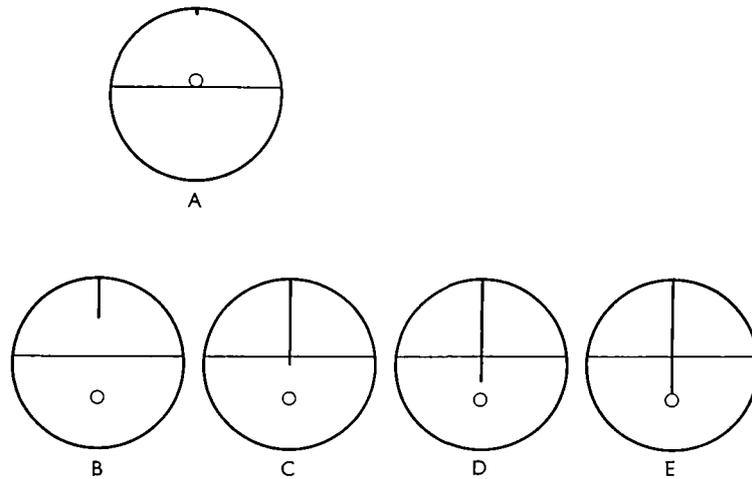by about 1·0, 0·3, 0·1 and 0 mm (Fig. 4B–E). Samples taken from each stage were

Fig. 4. Donor eggs for testing the cytoplasm beyond the furrow tip in stages indicated in the figure. Distances from furrow tip to the point for testing: A, 1·0 (mm); B, 1·0; C, 0·3; D, 0·1; E, 0.
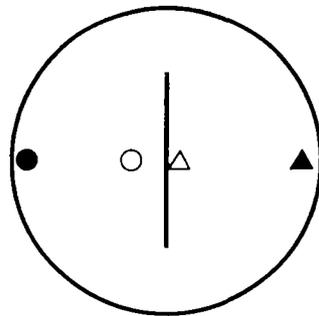

Fig. 5. Position of removal (O, △) and deposition (●, ▲) of cytoplasm for the transverse distribution of FIC perpendicular to a furrow.

Table 6. *The distribution of FIC perpendicular to the furrow*

Distance from furrow, mm	No. of cases	Results +	±	−
0·1	16	1	0	15
0	16	11	1	4

Symbols as in Table 1.

transplanted to recipient eggs at stage A of Fig. 4. In the vegetal hemisphere, FIC was found typically about 0·1 mm ahead of the furrow tip (Table 5).

Lastly, the distribution of FIC was investigated in a direction at right angles to the cleavage furrow to find the greatest width at which effective FIC could be obtained. The same egg with the furrow one third of the egg diameter served as the donor and recipient. Subcortical cytoplasm was taken from 2 regions, one directly adjacent to
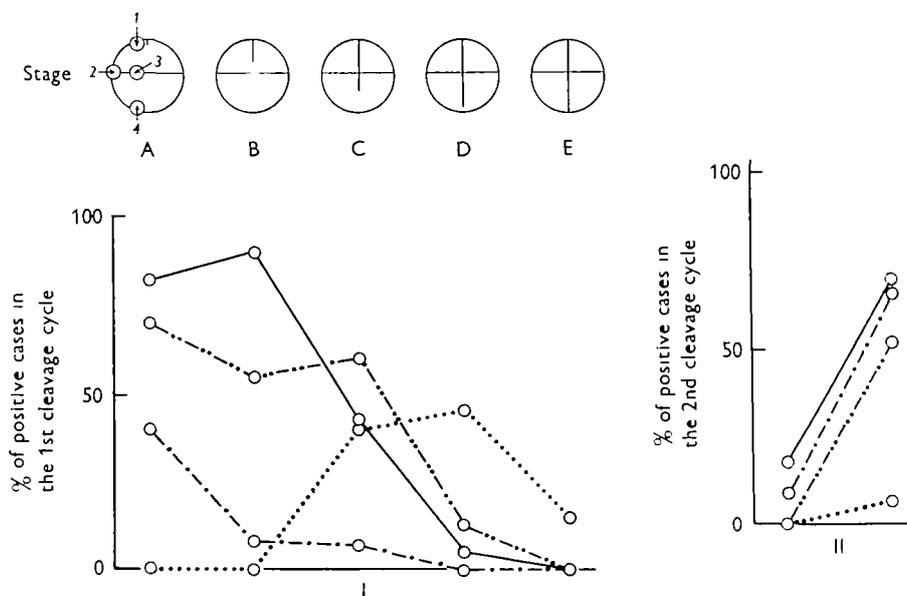
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Fig. 6. Percentages of successful furrow induction at 4 locations of transplantation at 5 different stages of development as shown at the top of the figure. I, Reaction during the 1st cleavage cycle. II, Delayed reaction in the 2nd cleavage cycle for operation performed at D and E of I. Ordinates, percentages based on 10–29 experiments. Abscissae, stages corresponding to A–E above. O— · —O, animal (*1*); O———O, lateral (*2*); O— · · — · · O, median (*3*); O · · · · O, vegetal (*4*).

the furrow and the other about 0·1 mm to the side of the furrow (Fig. 5). The former induced a furrow in 11 cases out of 16; in contrast, only one positive case out of 16 was obtained with the latter (Table 6).

Reactivity of superficial layer to furrow induction by FIC

The receptivity of the cortex to the influence of FIC to form the cleavage furrow was recognized not only on the lateral equatorial region but over the entire surface of the recipient eggs. Such reactivity appeared intermittently in accompaniment with the cleavage activity.

In contrast to the studies in the first section of this report, where the state of the donor eggs was varied, in the second, attention was focused on the recipient eggs. In order to follow changes in the reactivity of the recipient to transplanted FIC, both the positions of transplantation and the stages of the recipient at the time of transplantation should be fixed. Stages selected are shown in the upper part of Fig. 6. These stages also serve crudely as a time-scale about 20–30 min apart. Places of transplantation were (*1*) animal pole region, (*2*) lateral equatorial region, (*3*) equatorial region near the first cleavage plane (median region) and (*4*) vegetal pole region (Fig. 6). FIC was taken from donors corresponding to the stage A to B of Fig. 6. One egg was used in testing each region at each stage so that altogether the experiments were performed in 20 combinations (5 stages × 4 regions).
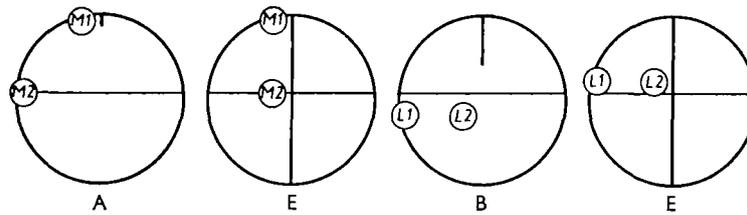
Fig. 7. Stages of recipient (defined by Fig. 6) and positions of transplantation for comparison of time of reaction between 2 transplants: $M1$ and $M2$ on the same median, $L1$ and $L2$ on the same latitude.

Table 7. *Comparisons of time of reaction between animal and equatorial regions and between lateral and median regions*

Stage and position of transplantation	No. of cases	Positive reaction at both regions on one egg	Comparison of time of reaction		
		Median			
			Earlier at animal region	Simultaneous	Earlier at equator
A Animal region $(M1)$	27	12	6	5	1
Equator $(M2)$	27				
E Animal region $(M1)$	29	15	13	2	0
Equator $(M2)$	29				
		Lateral			
			Earlier at lateral region	Simultaneous	Earlier at median region
B Lateral region $(L1)$	55	20	2	15	3
Median region $(L2)$	55				
E Lateral region $(L1)$	10	5	0	5	0
Median region $(L2)$	10				

Appearance and disappearance of the reactivity differ in time for each region. Data by stages of transplantation of recipient eggs are shown graphically in Fig. 6.

In the transplantation at the animal region (*1*), percentages of successful cases decreased as the stage of transplantation progressed from A to C. At stages D and E, the animal cortical reactivity for the first cleavage cycle was completely lost. However, some of the cases which failed to react for the first cleavage did react after the start of the second cleavage as shown in Fig. 6, II. In short, lower percentages shown by transplants in the animal region were due to the fact that the reactivity here was already fading even at the stage A.

In the transplantation at the lateral (*2*) and median (*3*) regions in stages A and B, all furrows induced appeared after the natural furrow traversed the same latitude as the transplants. Some negative cases in the transplantation at stages D and E reacted after the second cleavage furrow reached the same latitude as the transplants (Fig. 6, II).

In the transplantation at the vegetal region (*4*), positive cases were obtained only as late as stage C, in which the reactivity appeared after the furrow tip reached the

Table 8. *Transplantation of FIC to egg before the first cleavage*

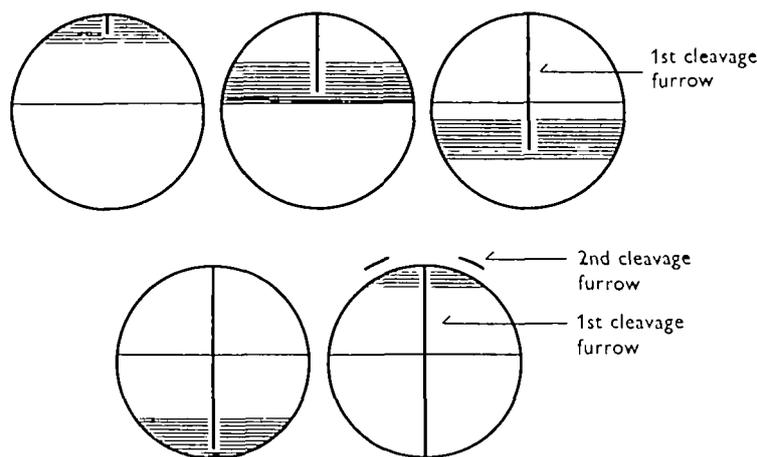| Time before the first cleavage, min	No. of cases	Results		
		+	±	−
$40 \geqslant T > 0$	13	8	3	2
$T > 40$	22	1	3	18

Symbols as in Table 1.

Fig. 8. Relationship between the progress of the cleavage furrow and the site of competence of the cortex to react FIC (═══).

vegetal pole. Percentages at the vegetal region increased as the stage of transplantation progressed from C to D, followed by a decrease. But among transplants made at stage E, few cases reacted after the second cleavage furrow reached the vegetal pole (Fig. 6, II).

Generally speaking, numbers of positive cases reacting to the second cleavage increased as the stage of transplantation was delayed. Differences in reaction must depend on the length of the time for which FIC retains the inductive capacity after deposition; this will be taken up later.

To compare more precisely the time of reaction on the animal and equatorial regions, 2 transplants were given to a single egg; in one group 2 transplants were made medially at the animal and the equatorial regions (Fig. 7, M 1, M 2), and in the other at 2 regions on the same latitude (Fig. 7, L 1, L 2). The stages of recipient used in the former group were A and E of Fig. 6, and in the latter, B and E. These are collectively shown in Fig. 7.

When FIC was transplanted at the animal and the equatorial regions simultaneously at stage A, altogether 12 cases were obtained in which the furrows were induced in both regions. Out of the 12, in 6 cases the reactions occurred earlier on the animal region, in 5 cases approximately simultaneously and in only one case, earlier on the

equatorial region (Table 7, Median). In similar transplantation performed at stage E, 15 cases of both positive were obtained in the second cleavage. Animal region reacted earlier than the equator in 13 cases out of the 15, and in the rest, approximately simultaneously (Table 7, Median). All the positive reaction took place after the furrow tip passed through the respective latitude.

In the transplantation at 2 places on a latitude at stage B, 20 cases were obtained in which FIC induction appeared in 2 regions. Reactions occurred approximately simultaneously in 15 out of the 20 (Table 7, Lateral). Of the remaining 5 cases, in 2 cases lateral region reacted earlier, and in 3 the reverse. In the same transplantation done at stage E, in all cases which were positive on both areas, reaction took place approximately at the same time as the second cleavage furrow reached the same latitude as transplants (Table 7, Lateral).

Lastly, the transplantation was made into the eggs before the first cleavage. The tests were made in 2 ways; in one, transplantation was made within 40 min before the onset of the first cleavage and in the other, still earlier. FIC was put under the cortex of the animal pole region of uncleaved fertilized eggs.

In the former, the furrow was induced in 8 cases out of 13. In the latter test, only 1 case out of 22 was positive (Table 8). All the reactions were observed just after the first cleavage furrow appeared on the animal pole region (see Fig. 9 A). Results showed that FIC remained effective for about 40 min.

The results of the second section of this work are summarized as follows: the region of the cortex which responds to FIC travels as a belt medially from the animal pole towards the vegetal region with the advance of the cleavage furrow of the host egg as illustrated in Fig. 8.

DISCUSSION

From the results of the present paper, it is beyond doubt that 2 separate factors take part in furrow formation in amphibian eggs. One is the subcortical component (FIC) which induces the cleavage furrow in the cortex, and the other is the cortex which is competent to react towards FIC to become a cleavage furrow.

Concerning the behaviour of the latter factor, it has been described in terms of intermittency of the reactivity of the cortex. However, the present results do not completely exclude the possibility that the cortex has no intermittency of its own for acquiring and losing reactivity; instead, the transplanted subcortical component may be activated every time the main furrow of the recipient egg comes down to the same latitude as the spot of transplantation. If such a situation exists, it will also give rise to the intermittency observed. Attempts at distinguishing between the 2 possibilities are being carried out.

Zotin (1964) reported, in amphibian eggs, that the dispersion of the diastema by D_2O brought about many furrows at several places where the diastema fragments were located. He interpreted the fact that the diastema material stimulated the cortex to contract which resulted in the formation of the furrows. However, the idea of furrow induction by the diastema is applicable only to the animal half of the egg, since

the diastema is not found in the vegetal half. On the other hand, FIC can always be found at the subcortical region along the cleavage furrow through the entire process of furrow formation.

Arnold (1971) reported, in squid eggs, that cutting the base of a furrow destroyed the filamentous bands which, in turn, caused degeneration of the cleavage furrow starting from the cut towards the distal region. In the present experiments, when a large amount of the subcortical cytoplasm was removed from the bottom of the deepening furrow, the furrow was eliminated at the region of removal. Removal of the cytoplasm from other regions had no influence on the furrow.

In the unilaterally cleaving sand dollar eggs studied by Rappaport's group, a furrow could be made to appear even on the surface originally far distant from the mitotic apparatus, if the cell surface was pushed into the interastral zone (Rappaport & Conrad, 1963; Rappaport & Ebstein, 1965). According to Rappaport, bias stimulation from both asters is a cause for the experimentally formed furrow. In amphibian eggs, since it is technically difficult to repeat Rappaport's scheme, the present author attempted to graft the cortex taken from various regions instead of pushing in the surface. Positive results have been obtained of which details will be reported in the next paper.

A point worth mentioning is that, regardless of into which part of the egg transplantation of FIC was made, the furrow was always induced after the natural furrow passed through the same latitude as the position of transplantation.

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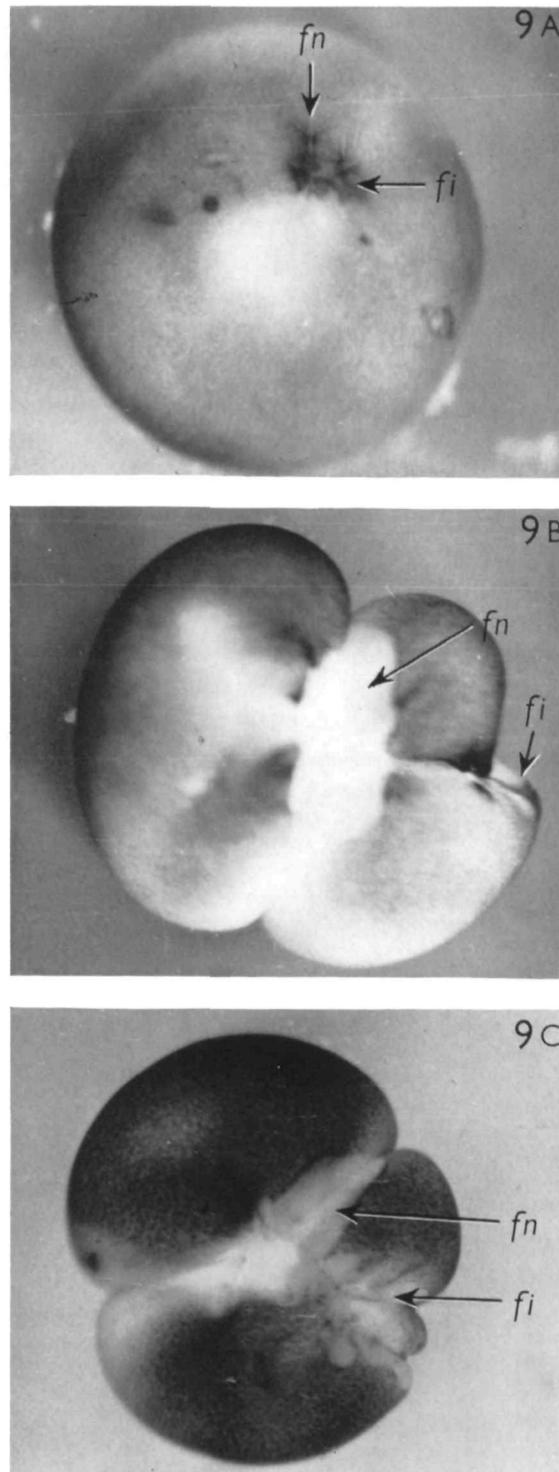
Fig. 9. Photomicrographs of successful dent formation. *fi*, induced furrow; *fn*, natural furrow. A, Initiation of dent formation; B, well formed dent; C, pale surface along the side of an induced furrow obtained by linearly transplanting a large amount of furrow-tip cytoplasm.