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Insensitivity to cytochalasin B of surface contractions keyed to cleavage in the *Xenopus* egg

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SUMMARY

In fertilized eggs of *Xenopus laevis* a marked flattening of the pigmented animal hemisphere has been observed. The flattening begins 15–20 minutes before the appearance of the cleavage furrow. As the furrow develops, the pigmented surface relaxes and rounds up. The initial appearance of the furrow is thus shown to be a combination of furrow deepening and rounding up of adjacent pigmented surfaces.

It is demonstrated that the flattening is not caused by gravity or osmotic mechanisms and that internal pressure is increased during the flattening. The flattening is interpreted to be an isodiametric contraction of the pigmented surface. The contraction is not inhibited by injected cytochalasin B in sufficient concentrations to completely inhibit cleavage furrow formation.

These results are discussed with respect to the presence of two surface contractile systems, distinguishable on the basis of their differing sensitivity to cytochalasin B.

INTRODUCTION

In animal cells it has long been known that shape changes occur which are correlated with the cell division cycle. In cultured cells, the extended flattened shape, which is largely due to cortical structures, changes to a more spherical one. Such rounding up is probably not just a relaxation and assumption of a minimum energy configuration, because it is accompanied by active formation and withdrawal of surface blebs. Likewise, in sea urchin eggs, by the assumption of the spherical shape the cells can lift weight against gravity (Danielli, 1952; Schroeder, 1981) and in large amphibian eggs this can result in the raising up of egg mass against gravity (Selman, Jacob & Perry, 1976; Sawai, 1979).

Associated with the rounding up of an egg before division, is a general increase in firmness of the egg surface. This cortical change has been demonstrated in a variety of egg types by movement of cortical pigment granules in a centrifugal field (e.g. Zimmerman, Landau & Marsland, 1957) or by measuring the deformability of the egg surface (e.g. Selman & Waddington, 1955; Wolpert,

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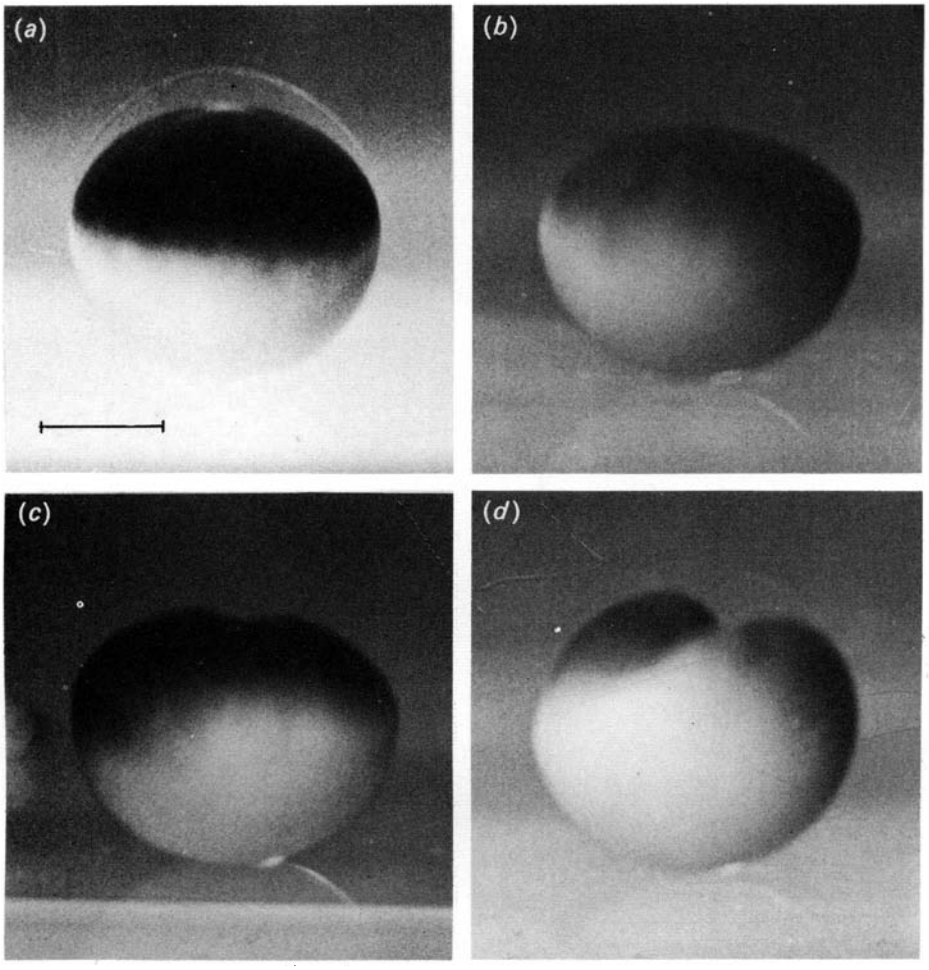

Fig. 1. Photomicrograph of a single fertilized *Xenopus* egg (*a*) undergoing flattening of the animal hemisphere about 15 min before cleavage (*b*) and the initiation of the cleavage furrow (*c* and *d*). All magnifications are the same. The bar line represents 0·5 mm.

1960; Sawai, 1979). The increase in firmness reaches a maximum just before cleavage begins and then a wave of decreasing firmness advances down the egg from the animal to the vegetal pole concomitant with the advance of the furrow.

Rounding up of cells in preparation for cell division and the associated hardening of the cortex have been shown to be necessary for cytokinesis to occur. Thus, if the more rigid cortical structures of sea urchin eggs are weakened or solated by increasing hydrostatic pressure or decreasing temperature, cytokinesis stops and reverses (Marsland & Landau, 1954). Such a physical interruption of the cytokinetic process is completely reversible. These experiments not only demonstrated the dependence of cytokinesis on the increased

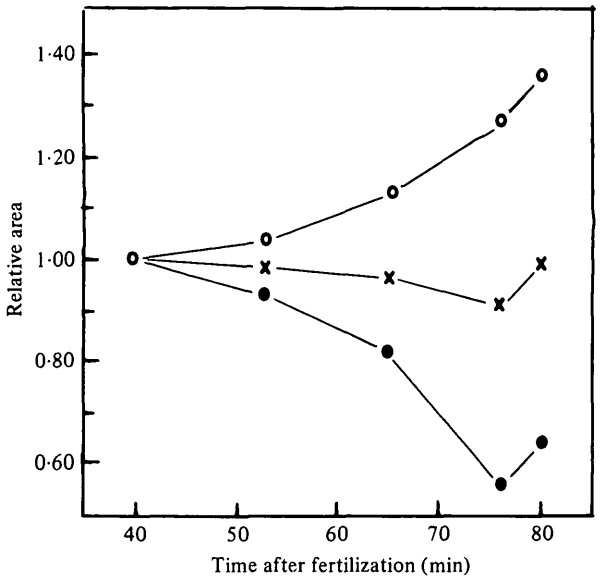

Fig. 2. Graphical representation of the changes in the surface area of the pigmented hemisphere (●—●), the unpigmented hemisphere (○—○) and the total surface (×—×) of a fertilized *Xenopus* egg in preparation for first cleavage. In this egg the furrow became apparent at about 75 min after fertilization.

cortical firmness but also showed that the cortical change is an endothermic process.

Some dynamic aspects of these surface activities have been recorded by time-lapse cinematography of slight deformations or pigment changes on the surface of amphibian eggs (Hara, Tydeman & Kirschner, 1980; Sakai & Kubota, 1981). In fertilized *Xenopus* eggs, a circular wave of surface pigment accumulation starts at the animal pole about 20 min before the first cleavage division and moves down the surface towards the vegetal pole at about 60 μm per min. When the cleavage furrow starts at the animal pole, its advancing tips move downward towards the vegetal pole, behind the wave of pigment accumulation and at the same velocity. The wave of surface pigment accumulation was also observed in an enucleate egg fragment (Hara *et al.* 1980; Sakai & Kubota, 1981) and was unaffected by concentrations of colchicine and vinblastine sufficient to block the succeeding cleavage furrow formation (Hara *et al.* 1980).

In this report, we describe experiments to see if the changes in the pigmented cortex of the *Xenopus* egg, prior to first cleavage, are in fact caused by cortical contractions. We show that surface contractions almost certainly occur and that they are not inhibited by cytochalasin B (CB) in concentrations sufficient to completely block the cleavage furrow which follows. We thus demonstrate two types of cortical contractions, distinguishable by their sensitivity to CB and by the form their contractions take.

Table 1. *The effect of egg position in the gravitational field on precleavage flattening*

Position in gravitational field	No. eggs	No. flattening	Flattening (%)
Upright	10	10	100
At right angles	10	10	100
Upside down	10	10	100

METHODS AND MATERIALS

Eggs of *Xenopus laevis* were obtained by injections of human chorionic gonadotropin into the dorsal lymph sac of adult females. Fertilization of freshly extruded eggs was accomplished by mascerating a testis from a recently decapitated male frog in a small volume of water and stripping eggs directly into this suspension. After fertilization, egg jelly coats were removed by swirling the eggs for about 30 sec in 35 mM-beta mercaptoethanol at pH 8·9. The eggs were then washed and held in 0·1 strength Ringer's solution.

Microinjection of eggs was done by the procedures of Gurdon (1973). About 80 nl of injection medium were injected through a calibrated micropipette. The injection carrier solution contained 88 mM-NaCl; 1 mM-KCl; 15 mM-Tris-HCl, pH 7·4. Cytochalasin B (CB) from Sigma Chemical Co., St. Louis, Missouri was dissolved at 10 mg/ml in dimethylsulphoxide. This stock solution was diluted with the injection carrier solution to give an intracellular concentration of CB of 10 μg/ml of cell water, assuming half of the cell volume to be water. After injection, eggs were placed into a healing solution containing 7·5 mM-tris-phosphate, pH 6·7; 50 mM-NaCl; 1 mM-KCl; 0·74 mM-$CaCl_2$; 0·82 mM-$MgSO_4$; 2·4 mM-$NaHCO_3$; penicillin and streptomycin at 10 mg/l each. Healing solution for eggs which had been injected with CB also contained CB at 10 μg/ml. After 20 min in the healing solution, eggs were placed in a viewing vessel for observation and photography.

Changes in egg shape were recorded photographically. Eggs were placed in 0·1 strength Ringer's solution in a glass vessel with optically flat, vertical sides. Pictures were taken with a Leitz macro lens either from directly above or from the side through a 45° angle, first-surface mirror. Negative images were projected onto paper through a photographic enlarger and the outlines traced for analysis. Changes in the surface area of both pigmented and unpigmented hemispheres were determined with a planimeter from side views. Confirmation of the planimeter results, and also calculation of partial cell volumes, were accomplished by the use of standard mensuration formulae, using the parameters taken from the traces. Both methods gave qualitatively similar results.

For experiments in which the effects of medium tonicity on egg shape changes were observed, the hypotonic medium was 0·1 strength Ringer's solution.

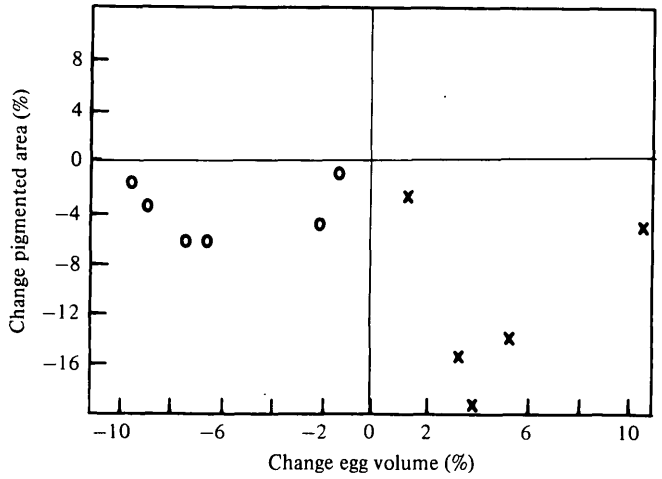

Fig. 3. The relationship between osmotically induced changes in total egg volume and the changes in the area of the pigmented hemisphere due to precleavage flattening. Eggs in hypertonic medium (○); eggs in hypotonic medium (×). For full description see the text.

Hypertonic medium consisted of Ringer's solution to which an additional 80 mM-NaCl was added.

RESULTS

While observing surface changes that occur in *Xenopus* eggs between fertilization and first cleavage, we noticed that there was a marked flattening of the pigmented animal hemisphere (Fig. 1 *a–d*). The change started about 15–20 min before the initiation of the first cleavage furrow and was usually accompanied by changes in the appearance of the pigment of the animal hemisphere. The furrow appeared, as usual, across the animal pole of the egg while the pigmented hemisphere was in the flattened condition. The flattening was more marked in some batches of eggs than in others. As the initial furrow deepened, the flattening of the pigmented surfaces on both sides of the furrow relaxed again into more nearly spherical shape.

We could envisage three ways in which this flattening could occur: (1) A localized softening of the cytoplasm could allow gravity to preferentially flatten the animal hemisphere, which normally is 'up' in the gravitational field. (2) Some sort of water withdrawal could preferentially shrink the relatively yolk-poor animal hemisphere. (3) There could be an active contractile event in or under the animal hemisphere cortex which caused a flattening of its surface.

The micrographs of Fig. 1 demonstrate the shape changes we are describing. In Fig. 2, we see plotted the quantitative changes in total egg surface, pigmented surface and unpigmented surface for a characteristic egg. Note that the area of the pigmented surface decreases as the animal hemisphere flattens and that

Table 2. *Changes in area of the pigmented hemisphere between fertilization and first cleavage in* Xenopus *eggs*

Fertilized	Injected	Sample size	% change in pigment area*
No	—	7	+1·0±1·0
Yes	—	5	−16·8±3·9
Yes	DMSO	5	−17·4±2·5
Yes	DMSO+CB†	6	−14·8±2·5

* % = (area pigmented hemisphere/total egg area) × 100,
% change = (initial % − final %/inital %) × 100 (M ± S.E.M.).
† CB = cytochalasin B; DMSO = dimethylsulphoxide in carrier solution.

simultaneously the unpigmented surface increases. An egg which is punctured at this time squeezes much of its yolky endoplasm out of the wound, demonstrating that there is a building pressure on the egg interior.

Characteristically, the expanding vegetal hemisphere does not completely compensate for the shrinkage of the animal hemisphere. As a result, the total surface and volume decrease a few per cent until the initiation of the first cleavage furrow when there is a relaxation and expansion of the flattened animal hemisphere on both sides of the developing furrow. The expanding pigmented bulges (Fig. 1*d*) then cause the total surface area and volume to return to the original values. The initial appearance of the furrow is thus due to both deepening of the groove and expansion of the animal hemisphere surfaces on both sides.

We initiated experiments to see if local softening of the egg could allow gravity to produce these shape changes. Fertilized eggs were drawn into glass capillary tubes whose inside diameters were slightly less than the diameters of the eggs. The cells were then oriented with the pigmented hemispheres either 90° or 180° to the original 'up' position. The results can be seen in Table 1. We concluded that simple gravitational effects could not produce these shape changes. It was also noted that the flattening could occur against the force of gravity.

We next tested the effect of changing tonicities of the medium on the observed shape changes. Eggs were placed in either hypo- or hypertonic media and changes in the pigmented hemisphere were again quantitatively monitored. The cells were photographed from a side view and the total cell volume and area of the pigmented surface were calculated from planimetry of the images (see Methods). The results are shown in Fig. 3. Despite the fact that *Xenopus* eggs are almost impermeable to water, we found that total egg volume did increase somewhat in hypotonic medium and decreased in hypertonic medium. In both cases, however, the pigmented hemisphere flattened and decreased in volume,

and cleavage looked normal. We concluded that shifts of water between the egg and the medium could not account for the shape change. It was noted that the animal hemisphere flattening could occur against the force of osmotic swelling.

From these observations, the flattening of the pigmented hemisphere was considered to be a contractile event near the surface of the pigmented hemisphere. Since actin-associated contractions have been observed at the surface of a variety of cells, and since actin has been observed in the cortex of the *Xenopus* egg (Franke *et al.* 1976), we decided to see if the observed contraction was inhibited by CB. Accordingly, we injected fertilized eggs with CB, healed the injection wound in medium containing CB and then observed their ability to undergo the precleavage flattening of the pigmented surface. The results are presented in Table 2.

There was little or no inhibition of flattening caused by the drug. Furthermore, no cleavage furrow appeared in the eggs injected with CB while controls injected with DMSO only showed normal cleavage. The inhibition of cleavage furrow formation by injected CB (see also Luchtel, Bluemink & de Laat, 1976) showed that the antibiotic was both active and inside the cell.

We conclude that the precleavage flattening of the pigmented hemisphere is a contractile event that is not affected by intracellular concentrations of CB which completely inhibit furrow formation. The contractile structure operates, at least most strongly, at or just under the pigmented surface of the egg. The differential sensitivity to CB of the surface contraction and the contractile ring of the cleavage furrow indicates that two distinguishable contractile systems reside in or near the pigmented cortex of *Xenopus* eggs.

DISCUSSION

We have interpreted the animal hemisphere flattening which occurs in fertilized *Xenopus* eggs just before first cleavage as a contraction of the pigmented surface. This interpretation was made because: (a) the deformation could not be accounted for by gravitational or osmotic mechanisms, (b) the deformation is toward a more energy-requiring shape and can take place against the force of both gravity and increased osmotic pressure, (c) the deformation results in increased internal pressure which is manifest both by a small but consistent decrease in cell volume as water is presumably squeezed out and by a squeezing out of endoplasm when a hole is made in the surface.

A contraction of the surface which is keyed to the first cleavage division is probably related to cyclic changes in internal pressure (rounding up) or surface firmness which have been shown in other systems to be keyed to the cleavage cycle (e.g. Danielli, 1952; Zimmerman *et al.* 1957; Wolpert, 1960; Sawai & Yoneda, 1974; Selman *et al.* 1955; Selman *et al.* 1976; Sawai, 1979; Sakai & Kubota, 1981; etc.). In all cases, surface firmness, or internal pressure, increases before the initiation of the furrow and then decreases as the furrow

forms and deepens. Similar surface changes occur during polar body formation in frog eggs (Kubota, 1967) and starfish eggs (Nakamura & Hiramoto, 1978). Marsland & Landau (1954) have demonstrated that such changes in surface stiffness are obligatory requirements for normal cleavage in sea urchin eggs.

By means of an elegant system of measuring surface deformability (firmness) at two different points simultaneously, Sawai & Yoneda (1974) have shown that a wave of cortical firmness starts at the animal pole and progresses as a broad circumferential wave towards the vegetal pole in a newt egg. This wave coincides both with a wave of pigment accumulation, as seen in the *Xenopus* egg (Hara *et al.* 1980; Sakai & Kubota, 1981) and with a wave of cortical reactivity to furrow-inducing cytoplasmic factors (Sawai, 1972). It also coincides in location and rate of movement with the tips of the advancing cleavage furrow.

In light of these observations in other laboratories, we suggest that the contraction of the pigmented surface that we have observed would probably be manifested as both increased surface firmness and as a wave of pigment accumulation by the other means of analysis (Hara, 1971; Hara *et al.* 1980; Sawai, 1979). The relaxation of contraction that we observed after furrow formation probably coincides with the downward movement of a broad band of contracted (stiffened) cortex towards the vegetal pole, along with the tips of the advancing furrow (Sawai & Yoneda, 1974). The timing of all of these events around the formation of the furrow coincide rather well.

If this analogy is correct, we have demonstrated that the waves of surface activity, manifest as 'surface contraction waves' (Hara, 1971; Hara *et al.* 1980) or waves of stiffness (Sawai & Yoneda, 1974), are caused by a surface contractile mechanism which is different from that of the furrow formation by virtue of its insensitivity to CB.

It is interesting to consider the nature of the CB-resistant contraction which we have demonstrated. Since it seems to be located in or near the *Xenopus* egg cortex and since actin has been located in the *Xenopus* egg cortex (Franke *et al.* 1976), we may speculate that the contraction might be based on an actin–myosin system. If this is so, there could be two types of actin-based contractions. One type would require actin microfilamentogenesis and would be sensitive to CB (Brenner & Korn, 1979; Lin, Tobin, Grumet & Lin, 1980; MacLean & Pollard, 1980). This type would be represented by the contractile ring of cytokinesis. The other type would presumably not require actin microfilamentogenesis since it is not sensitive to CB. The surface contractions associated with cleavage that we have described would represent this latter type. It is, of course, possible that the second type is not an actin–myosin system at all. Further work on this question is underway in this laboratory.

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