On the Rate of Movement of the Cleavage Stimulus in Sand Dollar Eggs

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ABSTRACT The rate at which the cleavage stimulus moves from the mitotic apparatus to the cell surface was calculated from measurements made on flattened, fertilized *Echinarachnius parma* eggs with excentric nuclei. The time interval between the appearance of furrows in the closer and more distant equatorial margins is proportional to the difference in the distance between the axis of the mitotic apparatus and the two surfaces. The calculated rate of stimulus movement is 6.3 ± 1.8 μ/minute. This rate is slower than that of free diffusion, and the usually studied examples of cytoplasmic streaming; it approximates that reported for microtubule growth.

The cleavage mechanism is established in the egg surface by the mitotic apparatus. Although this relationship was postulated (Ziegler, '03) and demonstrated (Yatsu, '12; Conklin, '17) many years ago, it has only recently been subjected to systematic experimental analysis (reviewed Rappaport, '65, '71b; Hiramoto, '71). It would appear that the changes in ultrastructure (Schroeder, '68; Arnold, '68, '69; Szollosi, '70; Selman and Perry, '70), mechanical properties (Chambers, '38; Hiramoto, '68, '70; Rappaport, '67; Wolpert, '66) and behavior (Rappaport, '69b) that characterize the narrow band of surface at the furrow base are elicited by a modification of the local subsurface environment that can be accomplished in cleaving eggs by the asters alone (Rappaport, '61; Hiramoto, '71). This modification has been termed a stimulus, but its nature and mode of movement are unknown. It could be achieved by transfer of substance to the equator, or removal of substance from the equator, or propagation of molecular change without transfer of substance (Rappaport, '65, '71b).

Regardless of its nature, it is apparent that the stimulus is conveyed from the axial region of the cell via the mitotic apparatus to the surface. Information concerning its rate of movement would therefore be useful in understanding the stimulation process. All other things being equal, equatorial surface more distant from the axis of the mitotic apparatus should form furrows later than similar surfaces located closer to the mitotic apparatus. It follows that the time interval between the appearance of furrows in the closer and more distant surfaces should be proportional to the difference in the distances between the axis of mitotic apparatus and the two surfaces. From this relationship the rate of stimulus movement can be simply calculated. The most convenient experimental material for these measurements is the flattened, fertilized egg in which furrows appear only at the margins (Yatsu, '08; Dan, '43; Rappaport, '68). The appearance of a furrow on the closer margin indicates that the stimulus mechanism is operative and the cell surface is capable of response. The only difference between the near and distant margin is their geometrical relationship to the mitotic apparatus.

The investigation revealed that the expected proportionality exists and that the rate of stimulus movement is 6.3 ± 1.8 μ/minute.

MATERIALS AND METHODS

Gametes of the sand dollar, *Echinarachnius parma*, were obtained by injection of 0.5 M KCl. Four minutes after fertilization they were denuded by expulsion through a 24 gauge hypodermic needle and thereafter were maintained at 19° or cooler.

Experiments were accomplished with Leitz micromanipulators in previously described (Rappaport, '68) simple operation chambers and observed and photographed through an inverted microscope. At the streak or early amphiaster stage (30 to 45 minutes after fertilization) eggs were flattened against the bottom of the operating chamber with the sides of two stout, blunt glass needles. The needles were positioned parallel to the long axis of the mitotic apparatus and on either side of it (fig. 1). As they were slowly lowered, they flattened the egg which retained its circular profile. All eggs were flattened to 175 μ diameter. At the same time that they compressed the egg, the needles were used to control the position of the mitotic apparatus by exerting pressure upon it from one side or by closely confining it between them.

Eggs were positioned so that the scale of an eyepiece micrometer was superimposed upon the spindle center and oriented at right angles to its long axis so that it was, in effect, superimposed on the equatorial plane. Measurements were made at $\times$ 400 magnification. The distances measured here were spindle-to-surface distances (Rappaport, '69a) which constituted the distance from the spindle center to the cell margin on a line passing normal to the mitotic apparatus. Measurements were repeated frequently as astral expansion sometimes altered the position of the mitotic apparatus shortly before furrowing. Furrowing was considered to have begun when the equatorial margin moved 5 μ toward the mitotic apparatus and continued to move. When the furrow developed on the near surface, a stopwatch was started and when the furrow appeared on the distant surface, the watch was stopped.

RESULTS

Typical eggs appear as illustrated in figure 1. The extension of the astral rays immediately before furrowing was notable but, unfortunately, the growth of individual rays could not be followed with confidence. It is apparent, however, in eggs with highly excentric mitotic apparatuses, that many astral fibers grow to lengths that would have been impossible were the mitotic apparatus central and the cell spherical.

The relation of the time interval between the appearance of the two furrows to the difference in distances between the two equatorial margins and the spindle

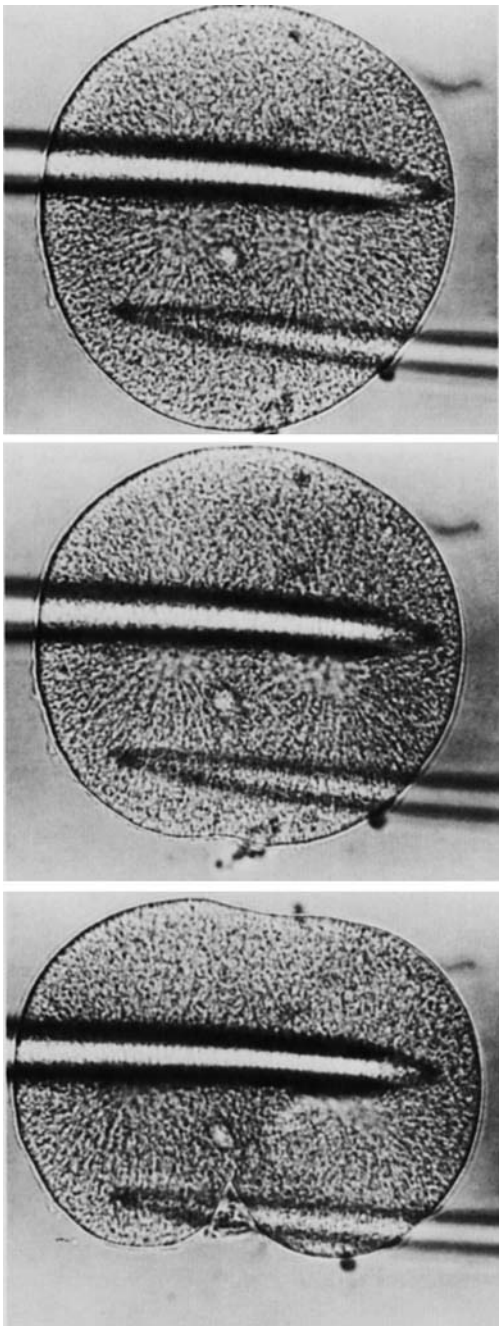

Fig. 1 Sequence of changes beginning at the upper photograph as furrows appear first in the near (lower) and then in the distant regions.

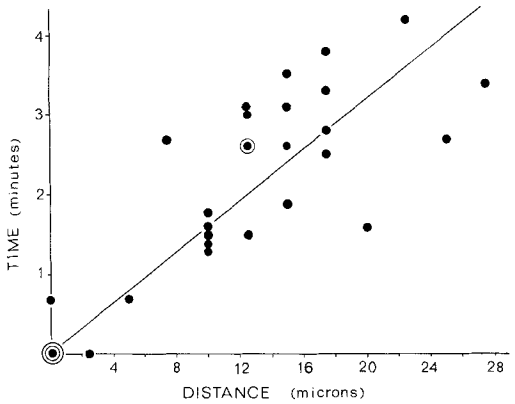

Fig. 2 The relation between the difference in distance between the spindle and the near and distant cell margin, and the difference in time between the appearance of the furrow in the near and in the distant margins.

are shown in figure 2. The relationship appears to be linear, and the rate is 6.3 ± 1.8 μ/minute.

DISCUSSION

The experimental design used in this investigation is based upon the geometrical relations found in the eggs of species with normally excentric nuclei. The use of artificially flattened eggs adds simplifying refinements, in that furrow formation is restricted to the cell margins and the position of the mitotic apparatus can be precisely controlled. The consequences of this manipulation, however, are apparently identical to those found in the egg of the Japanese sand dollar, *Astriclypeus mannii*, in which the nucleus is normally somewhat excentric and lies closer to the animal pole. In *Astriclypeus*, the furrow appears first at the animal pole, producing a temporarily heart-shaped egg (Dan and Dan, '47). Later a furrow appears in the vegetal region and then it deepens actively in the entire equatorial plane. The unilateral furrow characteristic of coelenterate eggs may be attributed to a geometrical circumstance in which the vegetal surface lies beyond the maximum distance that the cleavage stimulus can traverse. Production of unilateral furrows in eggs with normally symmetrical furrows, and symmetrical furrows in eggs with normally unilateral furrows has been accomplished by geometrical alterations that are consistent with this interpretation (Rappaport and Conrad, '63).

The stimulus, as described in the introduction, moves toward the surface at about 6 μ/minute, and its rate of progress may be compared with that of other previously described activities which could accomplish intracellular transport. Wolpert ('65) tabulated a series of measurements indicating that the rate of typical protoplasmic streaming in different kinds of cells is at least 20 times the rate reported here and more often 40 to 50 times faster. Hard and Cloney ('72) report that particles move centripetally toward the centrosome in interphase newt eosinophilic leucocytes at rates varying between 30 and 150 μ/minute depending upon particle size. Centripetal movement is mediated by linear elements, perhaps microtubules. They also report that the rate of particle movement away from the centrosome is about one-half that of the inward rate and much more variable, as it is apparently affected by cytoplasmic streaming accompanying ameboid locomotion. Classical cytological studies of dividing eggs and cells (reviewed Wilson, '28) revealed that, as the asters expand, larger cytoplasmic particles are displaced toward the cell periphery and equator. Whether the displacement is accomplished by cytoplasmic currents or by mechanical pressure of the radially expanding aster is unclear. Further studies of centrifugal movement in asters would be desirable.

Furrow establishment is immediately preceded by astral enlargement (Wilson, 1895). Swann's ('51) measurements of expanding asters at anaphase in polarized light indicate that, immediately before they achieve maximum size, their radius increases at about 4.5 μ/minute. In their investigation of reversal of Colcemid action, Aronson and Inoué ('70) found that established spindles elongated faster than 10 μ/minute following Colcemid inactivation. Ultrastructural studies of asters of invertebrate eggs reveal that they are composed of radially arranged microtubules interspersed with vesicular elements (Rebhun and Sander, '67, '71). Birefringence of the mitotic apparatus is attributable to the microtubules (Rebhun and Sander, '67) and the polarized light studies of growing asters and spindles cited above probably reflect microtubular growth (Bajer and Molè-Bajer, '72).

The cleavage stimulus moves more slowly than the examples of protoplasmic stream-

ing that are usually studied and more slowly than simple diffusion (Swann, '51). On the other hand, the rate of extension of birefringent elements in the asters and spindle approximates the rate of stimulus movement determined in this investigation, and this observation is consistent with others which suggest that the stimulus is associated with linear elements of the mitotic apparatus (Dan, '43; Rappaport, '68, '71a; reviewed Rappaport, '65, '71b). The observation that astral fibers can grow longer than the distance which normally separates the astral center from the equatorial surface (which was also figured by Harvey, '35) suggests that there is ample opportunity for contact between the fiber and the surface. Such contacts were described by Inoué and Dan, '51.

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