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Shape and Force in Cell Division

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In a cell about to divide, the shape of the internal structures are already hinting at where force would be generated and how it would work once division activity is initiated.

In the first stage of division, force is supplied by the elongating spindle which is due to sliding among microtubules making up the spindle. An important feature of this stage is to set the direction of division.

In the second stage, after a shallow furrow is formed, arrays of actin fibrils are formed along the furrow which contract and bring the division process to completion.

In bioloby, examples of correlation between shapes of organs and their functions are innumerable. In the present report, correlation between shapeand force in physiological activity will be dealt with.

EQUAL DIVISION

From numerous accumulated observations, the internal structure of a cell which is about to divide is diagrammatically shown in Fig. 1. The spindle is located at the center, mounted by chromosomes on the equatorial plane and flanked by 2 radiate-shaped asters at both ends. In the equatorial cytoplasmic region, rays coming from the opposite asters cross each other. The latter

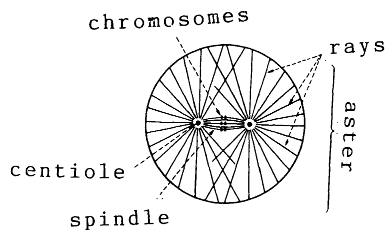

Fig. 1

point is important in later discussion. In Fig. 1, parts of the cell represented in black are gelated parts. As is well known, the spindle and rays of the asters are bundles of microtubules, polyserization products of tubulin molecules. The cell periphery is underlain by a gel layer of a few μ.

By looking at Fig. 1, one is tempted to imagine that if the 2 asters are pushed apart, the cell would divide. On the whole, this idea turns out to be true. But it must be pointed out here that, in spite of seeming simplicity of the idea, its effects on the cell surface are rather complicated. 2 polar surfaces (2 surfaces on bothsides as they appear in the Fig. 1) are pushed from inside, while the equatorial region is under 2 pulling forces to split it apart. The intermediate parts (upper and lower surfaces) lie between the 2 opposing forces.

Fortunately, it is possible to substantiate this notion by direct observations. Kaolin or animal charcoal particles are made to attach to the surface of a spherical cell before division. Then by selecting 2 particles on the circumference of the largest optical section of the cell, one can measure change in distance between the particles along the circumference, as the cell goes off the spherical stage through a cocoon and a dumbbell and finally to complete separation. (Dan, Yanagit & Sugiyama : 1937).

Since animal charcoal can be discerned on the upper surfaces facing observers, another set of data can be obtained in the direction perpendicular to the spindle axis. By integrating the 2 sets of data, area change of the cell surface can be calculated through division process. But the data along the circumference will suffice now.

Data taken in Mespilia globulus are shown in Fig. 2. Starting from the polar surface (Fig.2,A), the polar surface spreads in the middle period of division, but toward the end, it shrinks back, leaving some residual spreading. Since the last century, it was known that in the middle of the division, the astral rays joint to the polar surface spread and are parted into 2 bunches, just like when a paint brush is pushed against a stationary object. This so-called "fountain figure"

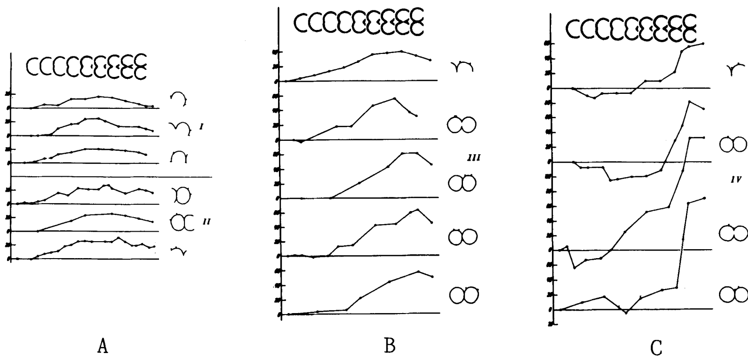

Fig. 2 A: polar surface, B: intermediate surface, C: equatorial surface

is not only a proof that the aster is being pushed against the cell surface but it is also an indication that the overlying surface must be spreading. When cleavage advances and furrow deepens, the stress on the aster slackens to some extent and the fountain figure gets less and the surface shrinks partly with residual spreading. The residual spreading is understandable because when a sphere divides into 2 daughter spheres without changing volume, surface area should increase about 26 %. The behavior of the intermediate surface (Fig. 2 B) is essentially similar to that of the polar surface except the degree of spreading is more than the latter since the intermediate surface is being subjected to 2 opposing forces.

On the contrary, the behavior of the equatorial surface is very different from others (Fig.2 C). At the end of division, the degree of stretching of this part far exceeds the preceding 2 regions, sometimes with no return. But before stretching, it invariably passes a phase of shrinkage. Why the surface which is to spread more than other parts must go through a shrinkage phase at the beginning is rather intriguing.

Concerning the above point, the author thinks the crossing rays in the equatorial region as a clue for solution. We consider a pair of crossing rays and 2 more rays, each ending close to the tips of the crossing rays. The latter 2 rays do

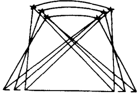

Fig. 3

do not cross as shown in Fig. 3. Since the tips of the rays.are embedded in the cortical gel, separate movements of individual tips are not allowed. As the result, these rays together with the spindle as a base make 2 unequilateral triangles. Then imagine that the base (spindle) elongates. While the apices of the triangles are drawn toward the base, they come closer to each other. This is actually the underlying mechanism of cell division shown by the following facts.

Kaolin particles are put on a spherical cell and 2 particles on future furrow region are selected. As the cell begins to change its shape, a series of camera lucida drawings are made on (1) cell contour, (2) positions of the 2 particles and (3) positions of the astral centers (tips of the spindle). There are 2 changing quantities in the drawings; deepening cleavage furrow and elongating spindle. The scheme of the present test is to see whether or not changing depth of the furrow can be accounted for on the basis of changing spindle lengths.

Testing is done as follows (Fig. 4): (1) on the 1st drawings, connect the 2 particles with the 2 astral centers. Thereby 4 segments of line making the sides of 2 unequilateral trianges are obtained. Now going to the 2nd drawing, (2) keeping the lengths of the segments unchanged, draw 4 circles using the segments as radii, placing the centers of the circles on new positions of the astral centers.

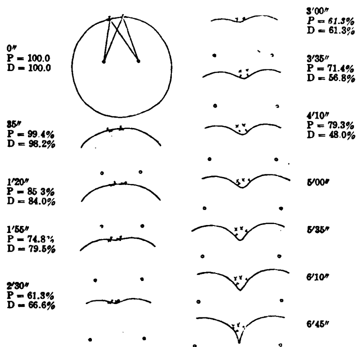

Fig. 4. V: kaolin particles, X: Intersections, O: astral centers P: distance between 2 particles as % of the initial value. D: distance between 2 intersections in % of the initial value.

2 intersection points of the circles give new positions of the apices of the triangles.(3) The same procedures are repeated

for succeeding drawings (Dan, K. :1943).

The results are rather satisfying down to the 5th or 6th step in Fig. 4 in which not only the depth of the furrow coincides with the intersection points, but also the degree of surface shrinkage agrees with that of intersection points.

However, as the division stage advances, when the surface shrinkage phase turns to a stretching phase (Fig.4, step 7), although distance between particles becomes longer, The intersection points come steadily closer. Moreover, at this very moment, intersection points begin to go off the furrow contour toward outside. This failure is not surprising since no measure is included in our scheme to separate the 2 triangles. This clearly means that although our scheme explains the initial phase of cell division nicely, another mechanism is required to bring the division process to completion.

In 1953, Swann and Mitchison, for the first time, reported a contradicting fact to our idea. They followed the state of the spindle and asters by their birefringence. By using birefringence it is possible to distinguish the metaphase and anaphase of chromosomes. If the egg cells are put in a relatively concentrated solution (0.5 M) of conchicine, the birefringence disappears in 3 minutes which can be taken as destruction of these structures. Now if colchicine is given at the metaphase, after disappearance of birefringence, the cell remained spherical and no division follows. If, however, given after the separation of chromosomes, the cells do cleave in spite of absence of the spindle and asters.

In 1956, Hiramoto came to a similar conclusion in a different experiment. He succeeded in sucking out the spindle and asters by a micropitette from sea urchin eggs. As in the colchicine experiment, if the spindle and asters are removed at metaphase no division follows, while if removed after anaphase, the cell can divide into 2. Both experiments indicate that there is a separate mechanism to bring division to completion which can be activated independently of spindle and asters.

Lastly, came Schroeder (1972). He was examining thin sections of sea urchin eggs by TEM (Fig. 5). In cleaving eggs

(with furrows), he found a girdle of fine filaments running along the bottom of furrow which appears as dark contour line in cross section. Later he identified these filaments as actin.

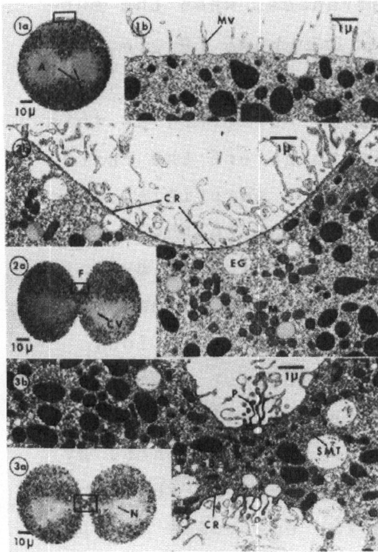

Fig. 5. Cross sections of cleaving sea urchin egg. 1a, 1b: future furrow region before division. Cell contour is a light line. 2a, 2b: furrow surface is lined by a darker line, meaning it is cross section of band of filaments. Note 2 ends are light showing the width of the filament band. 3a, 3b: complicate surface is covered by dark lines.

This looks to be the final molecular explanation for a constricting mechanism hinted by the preceding workers. But curiously enough, Schroeder cannot find the girdle of filaments as long as the cell remains spherical (Fig. 5, 1b). Therefore, although these theories can explain constriction perfectly, they fail to make the cell to start cleavage, nor can they anticipate in which direction the cell would divide.

Here comes in our theory of crossing rays. In embryonic development, the direction of division is rigorously fixed for each division and the spindle has a decisive role in setting the cleavage direction. Furrows by the crossing rays strictly bisect the spindle at right angle.

Summarizing, a furrow is started by our method but after the furrow is established, the constricting ring brings division to completion. Why the constricting ring waits to be formed until a shallow furrow is formed by the crossing rays and

how traction by the crossing rays is relayed over to constriction by the actin ring remains as an enigma.

UNEQUAL DIVISION

Contrary to equal division, the mechanism of unequal division is entirely different. The most basic feature of it is migration of resting nucleus to a predetermined corner of the cell long before nuclear and cell division (Dan: 1979).

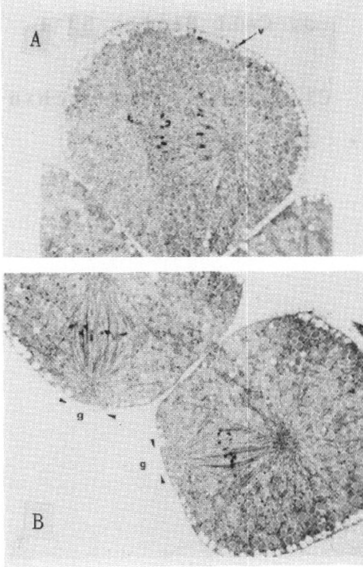

Fig. 6

In TEM studies of sea urchin eggs, the free (facing outside) cell surface is underlain by a row of vesicles (Fig. 6, A). The predetermined site can be recognized as a gap in the vesicular row. In the nuclear migration, 1 of the 2 centrioles invariably escorts the resting nucleus. When the differentiated site is reached, the centriole attaches itself to the site and anchors the nucleus to the cell surface (Fig. 6, B). This fixation of the spindle to an eccentric position in the cell makes possible for the cell to divide unequally. (Dan et al; 1983)

Although there are many examples of centriole leading nucleus or leading the movement of the entire cell, molecular mechanism of it is utterly unknown. Further study on this pointis urgently needed.

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