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SURFACE CHARACTERS OF DIVIDING CELLS IV. ALLOTMENT OF THE CORTEX AND PARTITIONING OF THE CYTOPLASM IN UNEQUAL DIVISION OF GRASSHOPPER SPERMATOCYTE UNDER THE TEMPERATURE GRADIENT

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ABSTRACT

The allotment of cell surface and the allotment of cell volume between two daughter cells were observed in unequal division of spermatocytes of the grasshopper, *Acrida lata,* caused by steep temperature gradient.

In applying a temperature gradient through the cell division, coincidence in position between the provisional division plane indicated by the tongue of mitochondria, and the actually formed furrow, has been established (i) by markers attached to cell surface and (ii) by calculated volumes of cytoplasm on the two sides of the planes.

On removing a temperature gradient while a cell is dividing, equality is regained as far as the volume of the incipient daughter cells are concerned but territories of the surface once allotted are strictly observed.

Unequal division, therefore, involves two different meanings; allocation of the surface, and apportionment of the cytoplasm both of which are dependent upon the development of asters.

INTRODUCTION

How the position of division furrow is determined in a cell entering into a division is a question of great interest.

In the normally unequal division of the grasshopper neuroblast, CARLSON (1952) approached this point by moving the spindle by a microneedle. If the spindle is moved from its typical eccentric position towards the center of the cell at the early anaphase, the neuroblast divides into equal halves correlating with the new position of the spindle. However, if the same operation is done at telophase, the cell divides unequally in conformity to the previous position of the spindle. Coincidence between the position of the spindle at anaphase and that of an incoming furrow shows that it likely is the anaphase when the

site of a future furrow is laid down. KAWAMURA (1960) reported that "the initiation of furrow is quite under the influence of an internal structure such as the mitotic apparatus, but any part of the cell cortex has furrowing capacity until the spindle body attaches tightly to the cell cortex at mid-cleavage stage". But since it was based on cells stretched drastically, a great deal of precaution may be required for a final interpretation.

In the previous paper (ISHIZAKA 1969), the normally equally dividing grasshopper spermatocyte was subjected to a steep temperature gradient. When a temperature gradient is applied along the spindle, the development of the aster on the warmer side is accelerated which, in turn, pushes the spindle towards the cooler side of the cell and the division becomes unequal. Before the cell departs from sphericity, the future cleavage plane is fore-shadowed by the tongue of the mitochondria.

In this paper, the position of division furrow will be studied from the view-points of both the allotment of the cortical material and of the allotment of volume between the daughter cells.

RESULTS

Allotment of Cell Surface

Attention is now directed to the movement of the cell surface, when the spindle is shifted and the ensuing division becomes unequal under the influence of a temperature gradient (ISHIZAKA 1969). Tracing of the movements of markers attached to the cell surface is done by the method described in the previous paper (ISHIZAKA 1966).

In Figure 1, four different conditions are illustrated, all having two markers (filled and open circles). The markers are lying on the side of a future furrow but more than the variance of control group (Table 1 in ISHIZAKA 1969) away from the bisecting plane of the spindle. Particular attention should be paid to filled markers which will mainly be dealt with in the following account.

Typical surface movement of the control cell (with no gradient) is shown in Figure 1C. Cell contours at metaphase and at the end of a division are represented by circles. This approximation is justified by the previous study (ISHIZAKA 1966). The positions of the two spindle poles are also shown by short horizontal arcs. At telophase, the daughter cells are equal in size and the markers remain on the same side respectively of the bisecting plane of the spindle throughout the division process. This means that the normal division of the spermatocyte is equal not only in terms of the volume but also in terms of the cell surface.

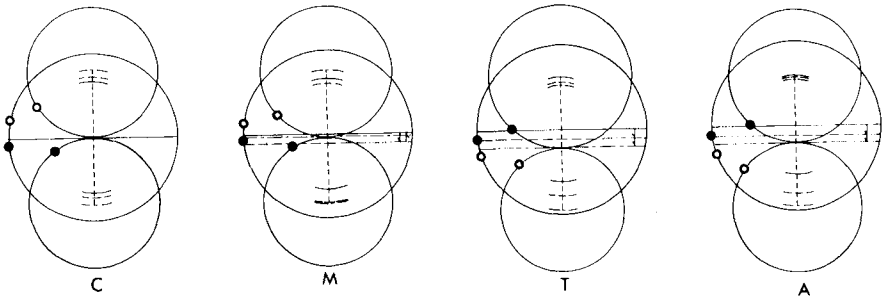

Fig. 1 Allotment of cell surface in cell division. Drawings of cell contours at the beginning and at the end of division under various conditions are superimposed in reference to the spindle axis. The positions of the bisecting plane of the spindle for the initial state is represented by real line and for the shifted metaphase position by broken lines and the anaphase position by chain lines. The positions of the spindle poles are shown by three kinds of arcs in the same way. Two markers near a furrow are indicated by circles, the one which is under a particular consideration is represented by a filled circle. C: The control. M: Treatment from prometaphase to metaphase. T: Treatment from prometaphase to telophase. A: Treatment from prometaphase to anaphase. (The upper side of the figure is warmer and the lower side is cooler. Temperature gradient, $6^{\circ}C/100\mu$).

The next case illustrated in Figure 1M is such that the gradient is applied through prometaphase and metaphase but is removed at the beginning of anaphase (corresponding to M-group of Table 1 in ISHIZAKA 1969). Under this circumstance, although the spindle is shifted for a while, before the furrowing begins, the spindle begins to return towards the initial position on abolishing the gradient. During such movements of the spindle, the bisecting line of the spindle which was initially on the upper side of the filled circle (real line) comes down below the circle at the end of metaphase (broken line) and goes back again nearly to the original place (chain line). As the result, after division, the filled circle is found on the lower daughter cell which has even regained the same volume as its sister cell on the warmer side so that the end result is practically the same as C-group. In other words, a temporary shift of the spindle during metaphase leaves little influence on the position of the furrow.

Jumping the anaphase which will be taken up later, application of the gradient through metaphase until the end of furrowing (corresponding to T-group of Table 1 in ISHIZAKA 1969) is next studied. Under a continuous application of a gradient, as illustrated in Figure 1T, the bisecting plane begins to move

down, passes the filled circle and continues to move to the extreme towards the cooler side so that the filled circle comes to lie on the upper larger cell on completion of the division.

As an intermediate case between T and M of Figure 1, the gradient is imposed, in the case of A, until late anaphase (corresponding to one of A-group of Table 2 in ISHIZAKA 1969). In this condition, the spindle is maximally shifted when furrowing begins. But one complication which differs from the previous case is that as the furrow continues to advance in absence of the gradient, the cytoplasmic volumes of the incipient daughter cells are being equalized. Because of this volume equalization, the bisecting plane of the spindle at anaphase (chain line) which was the most eccentric, becomes more central during telophase. But, since a furrow site is invariably determined by the bisecting plane of spindle at anaphase, it is accurately predictable to which one of sister cells a marker will go by its location in reference to the mitochondrial tongue at anaphase, notwithstanding the later volume equalization. In the end, therefore, the cell on the warmer side snatches more surface than that expected from the volume difference between the sister cells alone. Stating it otherwise, the allotment of the cortical material between the two sister cells is decided at the moment when the furrow site is determined at anaphase.

Allotment of the volume between sister cells

Degree of inequality of the volumes of two sister cells can be defined as the ratio, respective deviation of volume from the mean volume, ΔV, over the mean volume, $\bar{V}$. The cell volume can be easily calculated since the cell contour can be looked upon as a sphere.

Figure 2 shows relation between the degree of inequality of the volumes of the sister cells and the steepness of the temperature gradient when the latter is applied from spindle formation to the completion of division. It is clear that the steeper the gradient (abscissa), the more unequal are the volumes (ordinate). In an extreme case of the gradient of $6 \pm 0.3°C/100\mu$, inequality is 0.28 ± 0.04 which is more than five times the variance in the control.

In foregoing paragraphs, it was stated that a furrow is always formed at the site where the mitochondrial tongue comes in contact with cell periphery at late anaphase. Whether or not the ultimate allotment of the cytoplasmic volume between the sister cells is really determined by the partitioning by the tongue, is checked by comparing the volumes of cytoplasm of the resultant daughter cells with those on both sides of the tongue.

Inequality of the final allotment of the cytoplasmic volume between sister

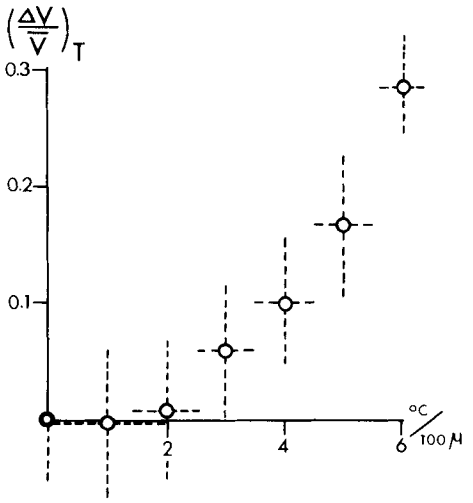

Fig. 2 Dependence of partitioning of volume between daughter cells $(\frac{\Delta V}{\bar{V}})_T$ on steepness of temperature gradient (°C/100μ) along spindle.

cells can be directly measured, which is $(\frac{\Delta V}{\bar{V}})_T$. Inequality of the partitioning of the volume by the mitochondrial tongue, $(\frac{\Delta V}{\bar{V}})_A$, corresponds to the shift of the tongue from the center of the cell. The shift was measured as the ratio (Tables 1 and 2 in ISHIZAKA 1969), the distance between the site of the mitochondrial tongue and the center of the cell, ΔR, divided by the mean radius of the cell, $\bar{R}$, i.e., $(\frac{\Delta R}{\bar{R}})_A$. From the data of the shift, inequality of the partitioning of the volume by the mitochondrial tongue is calculated by the formula,

$$(\frac{\Delta V}{\bar{V}})_A = \frac{3}{2}(\frac{\Delta R}{\bar{R}})_A - \frac{1}{2}(\frac{\Delta R}{\bar{R}})_A^3.$$

Correlations between $(\frac{\Delta V}{\bar{V}})_T$ and $(\frac{\Delta V}{\bar{V}})_A$ are shown in Figure 3.

The correlation under the continued application of gradient as illustrated in Figure 1T is perfect on the 45° line, assuring that the final allotment is a direct result of the partitioning by the site of the mitochondrial tongue. If the gradient is removed at late anaphase as illustrated in Figure 1A, the volumes of the incipient daughter cells are equalized during furrowing, although the furrow continues to advance along the plane predetermined on the circumference. Plots of such data are shown by crosses in Figure 3. They fall below the 45°

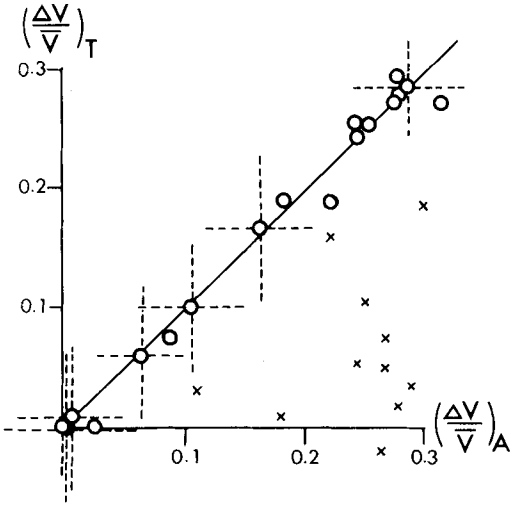

Fig.3 Relation between cytoplasmic volumes on two sides of mitochondrial tongue $(\frac{\Delta V}{\bar{V}})_A$ and the volumes of daughter cells $(\frac{\Delta V}{\bar{V}})_T$.
Open circles: Continued application of the temperature gradient. Crosses: Gradient removed at late anaphase.

line, meaning that the correlation decreases, or the volume discrepancy is being nullified. In other words, determination of furrowing site and the volume allotment of the cytoplasm are two independent factors.

Discussion

A few words will be spent on the relation between the surface and volume allotment. In the spermatocytes of grasshopper, when the spindle is shifted by applying or removing a temperature gradient, the surface allotment is determined by the bisecting plane of the anaphase spindle in whichever part of the cell the spindle happens to be. The result is analogous to that of CARLSON (1952) in the neuroblast. Such a plane foreshadowing a future furrow in the spermatocyte is structurally indicated by a tongue of mitochondria (ISHIZAKA 1969).

According to WOLPERT's theory (WOLPERT 1960), an unequal division is brought about by a local difference in the membrane tension. Since two parts of the cell must share the same tension, a part with a lower tension is expected

to bulge out acquiring larger curvature to be in balance with the other part with a higher tension and a smaller curvature. Therefore, by WOLPERT's theory, the allotment of the surface and that of the cell content are inseparable linked.

However, the author's foregoing observations definitely showed that the two factors are separable in grasshopper spermatocytes. If a temperature gradient is removed after the mitochondrial tongue reached the cell periphery, the surface is divided strictly by the position of the tongue although the cytoplasmic volume is being equalized.

In conclusion, unequal division requires twofold steps, i.e., allotment of the surface material and allotment of the cytoplasmic volume. It seems to the author that the surface allotment is decided by the mitochondrial tongue, while the base of inequality of the cell volume may be provided by inequality in the size of the two asters. A more detail analysis of the stresses during equal and unequal division will be published elsewhere.

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