PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES

Volume 26 April 15, 1940 Number 4

CYTOPLASMIC BEHAVIOR DURING DIVISION OF VACUOLATE PLANT CELLS

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Communicated March 11, 1940

The factors which determine the plane in which a meristematic cell divides must evidently be very important in controlling direction of growth and thus the development of form. In plant cells the orientation of the chromatic material, and particularly the distribution of the chromosomes at metaphase, give an indication as to where the plane of nuclear division is to be. Little evidence has been obtained, however, from the structure of either nucleus or cytoplasm in early mitosis which indicates where the cell itself is to divide, although Bowen[1] and others have shown that in certain cells the chondriosomes assume a characteristic position with reference to the future axis of the mitotic figure. Most of the plant cells in which mitosis has been studied are relatively small ones, which are rich in cytoplasm, and in which large vacuoles are absent. Cells which are larger and strongly vacuolate and yet are still dividing, and which in the aggregate are perhaps even more numerous than the more "typically" meristematic cells, have been largely neglected by cytologists. A study of cytokinesis in such cells makes it clear that the plane of the next division, and indeed the exact location of the future cell wall, are indicated by the distribution of the cytoplasm at a stage much earlier than one where these facts can be determined from nuclear orientation.

In such studies it is evidently necessary to observe cells in which the divisions are all in the same plane and in which the position of each new wall can therefore be predicted with some certainty. Such cells may be found in various parts of the plant, notably in the "rib" meristems of the young pith and cortex of the stem, where the divisions are all transverse to the axis. Even more favorable material is provided by the secondary meristems which are induced in fundamental tissue as a result of wounding. Here the new walls are all essentially parallel to the wound surface, and there is the added advantage that in the early divisions, at least, these new division walls in adjacent cells are usually directly opposite each other, so that the future position of the wall in a dividing cell may often be predicted very definitely. The cells are also relatively much larger than in

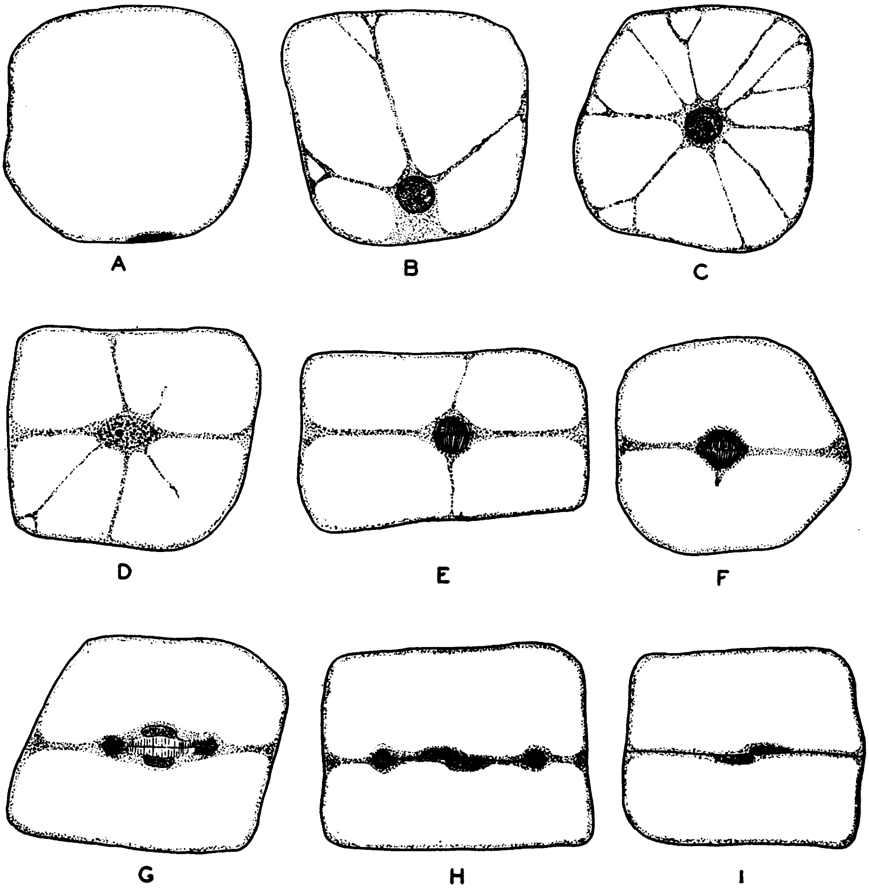

FIGURE 1

Semi-diagrammatic drawings of cell division in pith cells of *Ricinus*, which has been induced by wounding. The wound surface is parallel to the bottom of the page. *A*, resting stage; *B*, early prophase, enlarged nucleus migrating toward center of cell; *C*, prophase, showing beginning of formation of equatorial plasma strands; *D*, late prophase, with well developed phragmosome; *E*, metaphase; *F*, anaphase; *G* and *H*, telophases, showing development of young wall which follows the course of the phragmosome; *I*, two complete daughter cells.

ordinary meristematic tissue, since fully grown cells become dedifferentiated and meristematic, and the processes of cell division, particularly cytokinesis, may thus be seen in them very clearly.

Transverse, radial and tangential sections through wound tissues in a number of plants were studied. In figure 1, *A–I*, are shown semi-diagrammatic drawings of nine stages in the division of large pith cells in *Ricinus* which had been induced to divide by wound stimulus. In every case the wound face, and thus the future direction of the wall, is parallel to the bottom of the page.

In *A* is shown a typical differentiated cell, with the nucleus greatly flattened against the wall, cytoplasm small in amount and the bulk of the cell consisting of a large vacuole. The first effect of the wound stimulus, shown in *B*, is an increase in amount of cytoplasm, a rounding up and enlargement of the nucleus, and its ascent, on a column of cytoplasm, toward the center of the vacuole. Strands of cytoplasm begin to be thrown out to the wall. In *C*, the nucleus is now suspended in the vacuole by strands of cytoplasm. These are for the most part random in direction, but, from the very beginning of this stage, some are always present in the plane of the future wall. In *D*, a later prophase, these equatorial strands have become much heavier than the others and are now fusing at their bases. The nucleus is no longer spherical but is somewhat extended in the plane of its future division. Often at this stage there is one cytoplasmic strand passing upward and another downward at right angles to the plane of future division. *E* is the metaphase and *F* the anaphase of division, the nucleus now being suspended chiefly by the rather heavy cytoplasmic diaphragm which marks the position of the future wall. Other strands have often entirely disappeared at this stage. In *G*, the phragmoplast at telophase is extending laterally, the cell plate being carried far beyond the limits of the original nucleus by kinoplasmic fibrils, in the manner described by Strasburger, Treub, Bailey and others,[2] the system in face view giving the appearance of a circle or "halo." It is signficant that the course of the kinoplasmosome follows the equatorial diaphragm of cytoplasm established from early prophase, and that the developing cell wall which is being laid down in this way, and which finally reaches the wall of the mother cell, thus coincides in its position with the cytoplasmic plate. Before this process is completed the daughter nuclei (*H*) have usually returned to the resting condition. In *I* are shown the two daughter cells with the new wall between them, which occupies the position determined by the strands of cytoplasm very early in division. All the cells figured show an early division after wounding, and later cells are necessarily smaller, but they divide in the same way.

An essentially similar cycle of mitotic changes was observed in wounded tissues of *Tradescantia, Kalanchoe, Bryophyllum, Coleus, Phaseolus, Petunia* and *Cucurbita*. The results were entirely confirmed by direct observation of living dividing cells in hand sections from wounded regions.

The distinctive feature of the mitotic process in vacuolate cells, where the amount of cytoplasm is necessarily small in proportion to the size of the cell, is that this cytoplasm, from a very early stage in division, tends to become aggregated into a series of strands, sometimes anastomosing into a diaphragm, which occupies the position of the future wall and which thus indicates, considerably before the nucleus has done so, where the plane of division is to be. For this plate of cytoplasm the writers propose the term *phragmosome.*

That the phragmosome maintains its original position from the earliest stages to final wall formation is indicated by the fact that in early divisions of wounded tissue, where the new walls form a continuous series so that the wall of one cell is exactly opposite that of the next, the phragmosome is formed in the plane where the wall must ultimately be laid down. Evidence from plasmolyzed cells also shows that the phragmosome is firmly attached to the wall.

A number of minor differences from the method here described may sometimes be observed. Thus in many cases the nucleus remains at or near one wall during division, and then the phragmosome and the wall which follows it are formed on only one side of the nucleus. Division is usually approximately equal, but it frequently happens that the nucleus and phragmosome take up a position nearer one end of the cell than the other, so that the two daughter cells are markedly unequal. The phragmosome and wall commonly lie straight across the cell but occasionally, especially when division is unequal or the nucleus lies near one corner, the partition may be curved from the start.

Normal meristematic tissues, especially at the tip of stem and root, in which mitosis has chiefly been studied, are not favorable material for observation of the phragmosome, since the cells and vacuoles are small. In tissues where the cells are still dividing but where rather large vacuoles have already appeared, as in regions some distance back from the growing point in the developing pith and cortex, or in the fundamental tissue of massive organs like the fruit, the plate of cytoplasm may readily be seen, and such cells divide in essentially the same manner as has been described for wound meristems. The phragmosome thus seems to be a characteristic feature of the division of cells which have rather large vacuoles, and a visible expression of the polarity of such cells.

The significance of the phragmosome lies chiefly in its indication that the factors which determine the plane of division of the cell act upon the cell as a whole and not upon the nucleus alone. From very early prophase the position of the new wall is visibly determined in the cytoplasm. Nuclear orientation may not at first agree with this cytoplasmic orientation, for the equatorial plane of the figure often fails to lie parallel with the phragmosome; and it is thus evident, as has frequently been observed, that the figure is moving or rolling about in the cytoplasm. At telophase, however, it always comes back to a position where the cell plate is parallel to the phragmosome. All this suggests that the establishment of the division plane may be effected first in the cytoplasm rather than in the nucleus. The phragmosome is also of significance in those problems which deal with the factors determining the relative position of cell walls in multicellular tissues. The question, for example, as to whether the new walls are oriented as liquid films would be, in response to surface tension, or whether quite different factors are here involved, will require a study of the forces acting upon the phragmosome from the beginning and not alone upon the growing wall which follows it. A function of the phragmosome is presumably that of providing the material from which the new wall is built, and it is therefore easy to understand why the developing wall should follow it so closely.

The failure of cytologists to recognize such a conspicuous structure as the phragmosome is probably due to the fact that students of cell division have concerned themselves almost entirely with small cells having few or very small vacuoles. A recognition of the presence of such a structure occurs in two papers by Hanstein[3,4] in 1870 and 1880 which have been generally overlooked by later workers. Hanstein studied cell division in vacuolate dividing cells of the pith of various plants, and he figured and briefly mentioned a plate of cytoplasm in the plane of cell division, but failed to realize its significance for cytology and development.

Summary.—A study of cell division in vacuolate plant cells shows that from very early prophase the cytoplasm tends to become aggregated into a plate of more or less fused strands, the phragmosome, which occupies the position where the future cell wall will be formed. The fact that the entire cell body rather than the nucleus alone appears to be concerned in establishing the plane of division and the location of the new wall in plant cells is of general importance in problems of development.

[1] R. H. Bowen, *La Cellule*, **39**, 123–156 (1929).

[2] Described and literature cited in G. Tischler, *Allgemeine Pflanzenkaryologie*, Berlin (1921–1922).

[3] J. Hanstein, *Sitzungsber. Niederrhein. Ges. Natur- und Heilkunde Bonn*, Sitz., 19 Dec. 1870. (Reprinted in *Bot. Zeit.*, **30**, 22–28, 41–46 (1872).

[4] J. Hanstein, *Bot. Abhandlungen*, **4** (2), 1–56 (1880).