Connexions Between Meiocytes in Plants

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It is well known that the processes of meiosis begin and run their course in a perfectly synchronized succession in all the mother-cells of the pollen in a particular pollen-sac or in all the spermatocytes of a given cluster in animals.

In a study of spermiogenesis in the cat, Burgos and Fawcet (1955) attributed to the various connexions between the spermatocytes and to the syncitial clusters that are formed by the spermatids two important aspects: the normal presence of the connexions during meiosis, almost up to the moment of differentiation into spermatozoids, and their possible function as causes of the synchronization observed in the processes through which the cells evolve. Later studies (Fawcet et al. 1959, Postwald 1967) under the electron and phase-contrast microscope extended these observations and deductions to a wide range of invertebrate and vertebrate animals, including man.

Heslop-Harrison (1965) has mentioned the existence, before meiosis begins, of plasmodesms between cells forming the endothecium and the tapetum and between cells belonging to the tapetum and others coming from mother-cells of pollen grain, and, later, of wide cytoplasmic channels connecting the meiocytes, now completely separated from the tissue of the tapetum that surrounds them.

These observations of the connexions to be found in meiocytes, in both natural kingdoms, seem to point to the existence of a single system capable of establishing conditions of synchronization in the development of the cell processes that produce gametes.

This paper describes the connexions observed in the cells from which grains of pollen come in the prophase of meiosis and discusses a possible theory which would explain their formation and their importance in the synchronous development of pollen grains in a given pollen-sac.

Material and methods

At different periods of development anthers of Scilla nonscripta and Allium cepa were obtained and tested with light microscopy. One of the six anthers from each flower was stained in acetic orcein to estimate its state of development, while the remains were fixed in permanganate (KMnO₄, 2%,
in tap water) 2 hours at room temperature. The fixed anthers were dehydrated in acetone series and embedded in Durcupan ACM (Fluka). During the dehydration the material was stained overnight in lead-uranil acetate dissolved in 75% acetone for contrast enhancement. (Giménez-Martín et al. 1967).

To obtain the ultrathin sections a LKB Ultratome was used. The observations were made with a Siemens Elmiskop I and the pictures were taken on Scientia Gevaert plates.

**Observations**

*Cell-systems in meiocytes.* The cytoplasm of the meiotic cell is bounded externally by a typical plasmalemma consisting of a membrane unit 75Å thick. The plasmalemma is uniform throughout its length, without observed vesiculations or indentations or salients to make its surface heterogeneous. It forms a surface boundary enveloping the cytoplasm of the meiocytes, an apparently continuous layer which links them all up through the connexions to which it forms an external covering, imparting a plasmodial unity to them in face of the external environment (Fig. 1).

The cytoplasm is seen to be closely packed with vesicles 0.1–0.2μ thick, generally of the same length as the tubules it contains.

The various membrane systems that appear regularly in the cytoplasm of the various types of vegetable cell — endoplasmic reticulum and Golgi bodies — are represented in the meiocytes, during all phases of division, by small scattered tubules roughly 0.1–0.2μ long with an inner space of about 150Å, but without the formation of the typical endproducts found in other kinds of cell. The long tubules or cisternae found regularly as representative of the E.R. and the piles of flattened cisternae, with lateral vesiculations forming the Golgi bodies were not observed in the meiocytes, their place being taken, generally, by more or less fragmentary bits of these formations. The nuclear membrane is observed to be noticeably sinuous and does not form a continuous layer round the nuclei (Fig. 1).

We also observe plastids and mitochondria in the meiocytes, but their structure shows and elementary laminar system, of pro-plastidial character in the former, and with no apparent capacity to synthesize starch, while the latter show few tubules and are strikingly primitive in appearance. These small organs and the membrane systems in the meiocytes seem to point to a very undeveloped form of their existence.

*Connexions between meiocytes.* The meiocytes often show extensive connexions between their cell-components and constitute veritable channels or connecting tunnels. The extension of the connexions varies considerably, and tubules of various dimensions, mitochondria, plastidia, basic cytoplasm, etc., have been observed in the areas of intercellular connexion linking up all the components of the cytoplasm between the neighbouring meiocytes (Fig. 3).
In some connexion areas, the tubules and vesicles can be observed so arranged as to give the impression of being about to move across them, or of having crossed them, under the influence of cytoplasmic currents. Two

Fig. 1. Meiocyte at prophase. The nuclear envelope can be observed partially broken up Δ—Δ. Several cytoplasmic connexions (c) ▲ with adjacent meiocytes are apparent.
differences occur between the connexions described in the spermatocytes and spermatids and those observed in vegetable meiocytes: their central position

Fig. 2. Morphology of the meiocyte cytoplasm during meiotic prophase showing the dispersion of the membrane systems in the cytoplasm.

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crossing the equatorial plane of the cell in the former case, while in the latter they seem to be arbitrarily placed; and the exclusively cytoplasmic

Fig. 3. a) Cytoplasmic and nuclear connexions (c); N=nucleus. b) Plastid lying across the connexion canal (P).
nature of the connexions in the case of the former, whereas in vegetable meiocytes we frequently find nuclear connexions. (Figs. 4 and 5). These show three enveloping layers: the plasmalemma and the double nuclear mem-

Fig. 4.
brane. It has frequently been observed that large portions of a nucleus may penetrate into the cytoplasm of the meiocyte next to it and the connexion that is established is reduced to a connecting channel that is small in pro-

Figs. 4 and 5. The nucleus (N) of one meiocyte appears connecting with the adjacent meiocyte; Cell-membrane (CM).
portion to the volume of the nucleus lying in the cytoplasm of the adjacent meiocyte. We have an example of this type of connexion in Fig. 6, a and b,

![Diagram of nuclear connexion](image)

Fig. 6. Seriated sections of a nuclear connexion. The great deal of nuclear material introduced in the adjacent meiocyte can be observed, in relation to the width of the connexion canal. N=nucleus; n=nuclear membrane; p=plasmalemma.

where we see the connexion cut tangentially and in its central region, the first showing the limit of the connexion at the level of the membrane and
the great bulk of the nucleus which is observed intruding upon the neighbouring meiocyte.

The frequency of the connexions varies, as do the dimensions. It is difficult to say how many connexions are established between neighbouring meiocytes owing to the sectioning technique used, which does not give complete and continuous runs of cell surface. The connexions are most often observed between those areas which have no callosa membrane and are in closer contact. As this membrane becomes thicker, the connexions gradually decrease in number and extension and eventually disappear, so that none have been observed at the beginning of metaphase I, where the meiocytes are seen free. In later phases of meiosis only connexions between callosa membranes may be observed sporadically, belonging to neighbouring cells, no connexions being observed between other cell areas.

Apparently the intercellular connexions which have arisen in the course of successive mitoses producing the mother-cells of pollen grains persist throughout all the stages of the prophase metaphase I and anaphase I of meiosis, and the final state of independence is re-established through the liberation of the plasmalemmas and the reabsorption of the connected nuclear masses.

**Discussion**

*Origin of the connexions.* The medial separating lamina comes into being during the mitosis of the meristematic cells through the activity of the Golgi bodies (Whaley and Mollenhauer 1962, Frey-Wyssling et al. 1963). These eliminate small vesicles from their cisternae, and the vesicles accumulate and arrange themselves and coalesce in the equatorial zone of the cell in a centrifugal direction, so as to form the medial lamina in the course of cytokinesis; and the cytoplasmic system itself seems to play its part working in a centripetal direction to produce the cleavage of animal cells after mitosis (Buck 1963).

Although we might indulge in all sorts of speculations in our attempts to explain the origin of the connexions, there is no doubt that they have been set up in the first case through incomplete cytokineses. Fawcet et al. (1959) expressed the opinion that the incomplete cleavage may be regarded as due to the difficulty that the membrane in course of formation has in breaking the fibres of the spindle that have accumulated along the axis of the cell as a result of the centripetal growth of the membrane. When these fibres break up, open communication is established between the daughter-cells, and this persists throughout cell differentiation. However, in vegetable cells, cytokinesis takes place in a centrifugal direction, and so in this case the last areas of the equatorial region to show fusorial fibres are those in contact with the cell membrane, and in these areas we might expect cytoplasmic connexions to remain. Therefore the observation that such connexions are not to be found in specific areas suggests that the mechanism whereby
they are set up may have had other origins.

An analysis of the cytokinesis of vegetable cells carried out by Risueño et al. (1968) used various substances and physical agents capable of partially or totally inhibiting cytokinesis and giving rise to more or less extensive or complete connexions between the cytoplasm of the two daughter-cells. Heat treatment likewise provoked partial or total inhibition of cytokinesis observed under both the light and the electron microscope. This inhibition was attributed to the effect of heat on the disconnexion of the cisternae that form the Golgi bodies, which are set free from one another and scattered all over the cytoplasm in isolation. This disconnexion led to considerably diminished production of vesicles and a shortage of them in cytokinesis, thus partially or totally inhibiting. Now, with meiocytes we find the Golgi apparatus showing natural characteristics similar to these artificially provoked by heat in the case of meristematic cytokinesis and we might therefore attribute the partial inhibition, in random positions, to a lack of Golgi vesicles due to their peculiar condition in meiocytes; and these positions would then set up the cytoplasmic connexions through the cell walls.

A diminished supply of vesicles and the marked slowness of some of the chromosomes in emigrating would thus establish the nuclear bridges linking the neighbouring meiocytes, but the significance of these is difficult to understand.

**Significance of the connexions.** Past speculation had attributed the synchronous development of cells in meiosis to two factors: in time, to a common origin or formation of the mother-cells; and in space, to their similar environment: two factors which would complement each other in synchronizing the processes of meiosis. However, the connexions observed between meiocytes with extensive communications entitle us to regard them as true connecting roads carrying small organs and parts of cell systems to and fro between neighbouring cytoplasms and forming routes for the transport of nutrients and stimuli, conferring unity on all the components of a pollen-sac. Several authors have attributed the synchronous course of mitotic or meiotic processes to this association between cytoplasms.

Treub (1871) wrote in a study of vegetable material: “Les noyaux d’une même cellule se divisent de préférence tous à la fois”. Differences of chromosome complement between nuclei in the same cytoplasm (Giménez-Martín et al. 1964) and incomplete cytokineses providing chromosomal and cytoplasmic bridges (González-Fernández et al. 1965) did not prevent the nuclei from dividing synchronously. The total or partial inhibition of cytokinesis or of successive cytokineses, when artificially induced, provides cells with a varying number of nuclei of the same or different chromosome complement. In the cases observed by these authors some connexion was required between neighbouring cells to synchronize the division of the various nuclei, but it did not matter how extensive the connexion was for this purpose. When
the number of nuclei in the same cytoplasm was comparatively large (4 or 8), mitosis could begin either asynchronously if the nuclei were unevenly distributed about the cytoplasm or synchronously if the distribution was regular; but in both cases the course of mitosis was finally synchronized. The regulation of the mitotic processes of the nuclei in the same cytoplasm with a view to synchronization was thought to be governed by interaction between nuclei by way of the cytoplasm. And it may be thought likely that the same is true in the case of the meiocytes of a single pollen-sac.

Summary

1. The prophases of meioses in a particular pollen-sac of the monocotyledons *Scilla non-scripta* and *Allium cepa* run a synchronized course and their protoplasts are joined together by connexions.
2. The connexions are of different dimensions and they may be of either cytoplasmic or nuclear character, or they may arise from bridges set up by cell organelles.
3. The connexions have been observed throughout the prophase of meiosis only. Their possible origin and the consequences are discussed.

References