THE FORMATION OF THE GENERATIVE CELL IN THE POLLEN GRAIN OF *ENDYMION NON-SCRIPTUS* (L)

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SUMMARY

The generative cell wall in the pollen grain of *Endymion non-scriptus* is formed, as in somatic cells, from a cell plate between the vegetative and generative nuclei. This wall curves around the generative nucleus, and fuses with the intine to enclose the generative cell. The generative cell is subsequently freed from the intine by the constriction of the generative cell wall between the generative nucleus and the intine.

INTRODUCTION

Until the use of the electron microscope in the study of pollen development, the status of the generative cell within the pollen grain has been a subject for controversy. Bopp-Hassenkamp (1960), using the electron microscope, confirmed Maheshwari's (1949) observation that, since the cytoplasm around the generative nucleus stains differently from the bulk of the pollen grain cytoplasm, it is a separate cell. She did not, however, consider that a wall was present between the two cells, but only a double plasma membrane. Larson (1963, 1965) also expressed this view. Sassen (1964a, b) considered that a wall is present, but he did not speculate on its mode of development. Maruyama, Gay & Kaufmann (1965) observed the deposition of the generative cell wall, and suggested that it was by the fusion of pectin vesicles. Permanganate fixation was used in their study, so that they did not see cell-plate microtubules.

The work reported here formed part of a study of the development of the pollen of the bluebell, *Endymion non-scriptus*, from meiosis to germination. The stages described occupy a very short period during the developmental sequence, occurring just before anthesis. Figure 1A is a diagram of the mature pollen grain.

METHODS

Buds at the appropriate stage of development were removed from the bluebell inflorescence, and the anthers were excised from the bud below the surface of the glutaraldehyde fixative, where they were cut in half to aid fixative access to the pollen.
There are six anthers in two whorls in the bluebell flower, and the development of the three anthers in the outer whorl is synchronous. One half anther from the outer whorl was removed, and an acetocarmine squash was prepared for reference purposes, while the remaining anthers were transferred to fresh glutaraldehyde.

Fixation was in an isotonic series. The first fixative was glutaraldehyde, 2.5%, in 0.1 M cacodylate buffer at pH 7.0. The solution also contained 0.01 M calcium chloride and a trace of teepol. After an hour in this solution at 4 °C, the anthers were rinsed several times in a solution in which the glutaraldehyde was replaced by 0.22 M sucrose, and post-fixation was at 4 °C in 1.0% osmium tetroxide, buffered to pH 7.0 with 0.1 M cacodylate, the solution also containing 0.22 M sucrose. Osmium fixation was allowed to continue for 30 min. After a distilled water rinse, dehydration was by a graded ethanol series. The anthers were embedded in Araldite (Glauert, 1962) containing no plasticizer, using 1:2 epoxy-propane as a vehicle for the Araldite. Thin sections, cut on glass knives, were mounted on naked copper grids, and stained in uranyl acetate (Huxley & Zubay, 1961) and lead citrate (Reynolds, 1963). A layer of carbon was then evaporated on to the sections to stabilize them, and they were examined in an AEI EM6 electron microscope.

RESULTS

The pollen of *Endymion non-scriptus* is two-celled at anthesis. Shortly before anthesis, the single-celled microspore undergoes an asymmetric mitotic division, one daughter nucleus, the vegetative or tube nucleus, remaining near the centre of the grain, while the other, the generative nucleus, comes to lie close to the spore wall. It is the generative nucleus that subsequently divides to form the two sperm nuclei that participate in the fertilization of the egg sac.

After mitosis (Fig. 2) a cell plate is formed between the two daughter nuclei (Fig. 3). At higher magnification (Fig. 4) the component vesicles, tubular arrays of endoplasmic reticulum, and cell-plate microtubules can be seen. The cell plate curves around the generative nucleus, and meets the intine, thus forming a hemispherical enclosure around the generative nucleus, the rim of the hemisphere abutting the inner layer of the spore wall, the intine, and fusing with it (Fig. 5). The first-formed wall is of irregular thickness, and is electron-transparent, with a discontinuous electron-dense core (Figs. 5, 9). The mean thickness of this wall is about 0.2 μ, and a radial section through the generative cell at this stage shows that the generative cell wall fuses at right angles with the intine (Fig. 9).

In the mature grain, the generative cell lies free in the vegetative cytoplasm, and is separated from the intine. The mechanism by which the generative cell becomes detached from the intine is unusual and interesting.

Shortly after the establishment of the generative cell wall, differences begin to appear in the organelle populations of the two cells within the pollen grain. In the vegetative cytoplasm, the amyloplasts become packed with starch, and osmiophilic lipid droplets are formed. These lipid droplets accumulate in the vegetative cytoplasm, lining the generative cell wall (Figs. 6–8), and they provide a useful marker for the
position of the generative cell wall, for it becomes difficult to see at later stages of development, when it is much thinner.

During the process of detachment of the generative cell from the intine, the generative cell wall becomes appressed to the intine, this appression progressing from the original junction of the generative cell wall and the intine, and extending inwards, in a ring, between the generative nucleus and the spore wall. This phenomenon is shown diagrammatically in Fig. 1, B–F, and can be seen in progress in Figs. 6–8. The region of fusion proceeds inward, like a closing iris diaphragm, pinching off a discrete sac of cytoplasm, containing the generative nucleus itself, and leaving a disc of generative cell wall appressed to the intine, marking the position occupied by the generative nucleus at the end of the mitotic division. Figure 8 shows this clearly; the generative nucleus has moved toward the centre of the pollen grain, and is enclosed by the generative cell wall. Around the outside of the generative cell, there can be seen a row of the osmiophilic lipid droplets, and a strand of them links those around the generative cell with a row lining the pollen wall, and marking the presence of the portion of the generative cell wall which has been left, attached to the intine. The generative cell wall becomes much thinner during this process, and by its completion,
the wall is only about 200 Å thick (Fig. 10). Figure 11 shows, at lower magnification, a portion of the generative cell wall where it is laid along the intine, together with trapped fragments of generative cell cytoplasm.

**DISCUSSION**

There is evidently considerable basis for controversy as to whether or not there is a wall around the generative cell. A glance at Fig. 10 would confirm, rather than refute, the suggestion put forward by Bopp-Hassenkamp (1960) and supported by Larson (1963, 1965) that there is not a wall, and that the two cells are merely separated by double plasma membranes. It is only when the developmental sequence is followed that it becomes clear that a wall is present, at least initially, between the cell membranes.

The composition of the wall has not been determined. The vesicles forming the cell plate (Figs. 3, 4) are electron-dense, and have a similar appearance to the calcium pectate of the cell plate in vegetative tissue. The wall thickens rapidly after its first formation, and it is electron-transparent. During the formation of the cellulosic intine, numerous microtubules are apparent, running in a plane parallel to, and close to, the surface of the intine, but none are seen during the phase of rapid thickening of the generative cell wall. There is, however, a peak in the quantity of endoplasmic reticulum during the formation of this wall (Fig. 4). During callose deposition in the meiocyte, there is a phase of marked activity in the endoplasmic reticulum (Angold, 1967). This suggests that callose may be involved in the structure of the generative cell wall, but lacmoid staining does not positively confirm this. Lacmoid, however, is not as sensitive as aniline blue fluorescence techniques although it is more specific, and as the wall is only 0.2 μ thick it is difficult to tell, in the light microscope, whether or not staining has occurred. Callose is certainly laid down on the generative cell wall at germination (this observation forms part of a further report). In the light of investigations by Heslop-Harrison (1966) and Heslop-Harrison & Mackenzie (1967) on the passage of labelled nucleic acid precursors through the callose into meiocytes, it is tempting to speculate that the presence of a layer of callose, which has been suggested by Currier (1957) to act as a sealant, may enable the generative nucleus to exert greater control over the generative cell, surrounded as it is by vegetative cytoplasm.

Whatever the composition of the generative cell wall, it undergoes considerable expansion and, in the process, becomes much thinner. The mechanism involved in pulling the wall inwards, between the generative nucleus and the intine, is not apparent. Microtubules are observed at the leading edge of the ring of fusion (Fig. 10) but there is no evidence that they are involved in the process.

The behaviour of the lipid droplets is interesting. They provide a useful marker, in that they indicate the position of the generative cell wall, and it is evident that, even when part of it no longer surrounds the generative cell itself, but lines the intine, there is still a preferential distribution of the lipid near to the wall.

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The generative cell of Endymion

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REFERENCES


(Received 13 December 1967)
Fig. 2. Anaphase of pollen grain mitosis, showing generative nucleus chromosomes (chr) at the edge of the grain. × 6250.

Fig. 3. The formation of the generative cell plate (gcp) which can be seen curving round the generative nucleus (gn). × 4750.
Fig. 4. Part of the cell plate (gcp) showing the constituent vesicles. Numerous profiles of tubular endoplasmic reticulum (ter) can be seen, and the region is transversed by large numbers of microtubules (mt). × 22,000.

Fig. 5. The completed generative cell wall (gce) separating the vegetative nucleus (vn) from the generative nucleus (gn). × 6,500.
Fig. 6. The generative cell shortly after the onset of the unfolding of the generative cell wall (gcw). The lipid droplets (l) can be seen lining the generative cell wall, in the vegetative cytoplasm (vc), and marking the extent of the generative cell wall against the intine. × 6250.

Fig. 7. The generative cell (gc) at the completion of the separation from the intine. Increasing quantities of starch (s) are apparent in the vegetative cytoplasm (vc) at this stage. (gn, generative nucleus.) × 3000.

Fig. 8. The generative cell (gc) has moved toward the centre of the pollen grain. A line of the lipid droplets (l) may be seen, linking it with those remaining on the portion of the generative cell wall appressed to the intine. Starch (s) is now packing the amyloplasts in the vegetative cytoplasm. × 5000.
Fig. 9. Generative cell wall (gctv) soon after its formation, separating generative cell cytoplasm (gc) from vegetative cytoplasm (vc). The wall is fused with the intine (i). \( \times 45000 \).

Fig. 10. Generative cell wall (gctv) during the separation of the generative cell (gc) from the intine (i). Microtubules (mt) are visible where the wall folds back. \( 45000 \).

Fig. 11. A portion of the generative cell wall laid along the surface of the intine (i). The process began at the point marked by the double arrow. Remnants of generative cell cytoplasm have been trapped between the generative cell wall and the intine (arrows). \( \times 15000 \).
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