CAPSELLA EMBRYOGENESIS: THE CHALAZAL PROLIFERATING TISSUE

PATRICIA SCHULZ
Department of Biology, Rosary College, River Forest, Illinois 60305, U.S.A.

AND W.A. JENSEN
Department of Botany, University of California, Berkeley, California 94720, U.S.A.

SUMMARY

Electron-microscope and histochemical procedures were used to study the development and breakdown of the chalazal proliferating tissue in *Capsella*. This tissue is formed by the enlargement of several layers of nucellar cells at the chalazal end of the embryo sac. When the embryo reaches the early globular stage these enlarged cells start to disintegrate, beginning with those immediately bordering the embryo sac and continuing until all have broken down. Characteristic ultrastructural changes accompany the development and breakdown of the chalazal proliferating cells. The mature cells form increased numbers of dictyosomes and large amounts of endoplasmic reticulum (ER). This is accompanied by a thickening of the cell wall. As the cells begin to break down, electron density increases, plastids become polarized in the cells, dictyosomes disappear and the ER is dispersed and fragmented. Plastids, some mitochondria, and pieces of ER appear to be digested in autophagic vacuoles. Cell disorganization is accompanied by an increased number of microbodies and multivesicular bodies per cell. Finally, the nucleus breaks down and the plasmalemma disappears. The end wall ruptures and releases intact mitochondria, ribosomes, and portions of degenerated cytoplasm into the endosperm. Histochemical changes accompany these events. Also discussed are the antipodals and the destruction of the proximal part of the chalazal nucellus by the expanding megagametophyte prior to the development of the chalazal proliferating tissue.

INTRODUCTION

During the early development of the *Capsella* embryo a group of large, elongated cells appears at the chalazal end of the embryo sac. These cells, which have traditionally been called chalazal proliferating cells, eventually break down and are absorbed by the embryo sac. Question has been raised concerning the origin and function of this tissue which has been interpreted by some as a proliferation of the antipodals (Holman & Robbins, 1940). The purpose of this report is to show that the chalazal proliferating tissue in *Capsella* is formed by the enlargement of several layers of chalazal nucellar cells. Electron-microscope and histochemical procedures were used to study the changes in structure and composition which accompany the development and breakdown of these cells. The first part of this paper deals with an earlier stage of embryogenesis and describes the antipodals and the degeneration of that portion of the chalazal nucellus proximal to the embryo sac which is crushed by the expanding megagametophyte prior to the development of the chalazal proliferating tissue. The second part concerns the development and breakdown of the chalazal proliferating cells themselves.
MATERIALS AND METHODS

Plants of *Capsella bursa-pastoris* (L.) Medic., the shepherd's purse, were grown in the greenhouse from seeds collected at the Botanical Garden, University of California, Berkeley. The 2 types of fixation used on tissue prepared for electron-microscope studies were (1) KMnO₄ and (2) glutaraldehyde followed by osmium tetroxide (GA-OsO₄). In the first, whole ovules were dissected from the silicles and fixed immediately in 2 % KMnO₄ for 15-23 h at 4 °C. The tips of some of the older ovules were excised before fixation in order to facilitate penetration of fixative and embedding media. In the second, ovules were placed in 6 % glutaraldehyde buffered by 0.06 M phosphate at pH 6.8 for 4 h at 4 °C. This was followed by a 1-h wash with changes of 0.06 M phosphate buffer and post-fixation with 2 % unbuffered OsO₄ containing 4 % sucrose for 15 h at 4 °C. Tissue fixed by both methods was dehydrated in a graded acetone series. The tissue was allowed to remain overnight in 70 % acetone to which 1 % uranyl nitrate was added. All material was embedded in Epon 812 and sectioned with a diamond knife on a Servall Porter-Blum Ultramicrotome. Sections were stained on grids with lead citrate for 1-2 min (Reynolds, 1963). Observations were made with a Zeiss EM-9 electron microscope.

Material prepared for the histochemical localization of nucleic acids was fixed in GA-OsO₄, embedded in Epon, sectioned at 2 μm and stained with azure B (Jensen, 1962). The periodic acid-Schiff (PAS) reaction was used for the localization of insoluble carbohydrate (Jensen, 1962) and aniline blue black for a general protein stain (Fisher, 1968). Tissue prepared for these latter 2 staining reactions was fixed in glutaraldehyde, embedded in Epon, and sectioned at 1.5 μm. Permanent mounts were made of material stained with azure B and PAS with Zeiss phase mounting medium L15. Temporary mounts of material stained with aniline blue black were made with glycerol to which 5 % acetic acid had been added. Observations were made with a Zeiss light microscope.

RESULTS

*Destruction of the proximal portion of the chalazal nucellus by the growing megagametophyte*

*Capsella* has a monosporic, 8-nucleate, 7-celled curved embryo sac of the polygonum type (Henry, 1958). The structure and composition of the synergids, egg and young embryo, including the suspensor and basal cell, are described elsewhere (Schulz & Jensen, 1968a-c; Schulz & Jensen, 1969). During the early development of the egg the nucellus is destroyed by the expanding megagametophyte except where it borders the chalazal end of the embryo sac (Henry, 1958). This brings the immature megagametophyte into direct contact with the inner integument on 3 sides. A portion of the chalazal nucellus immediately adjacent to the embryo sac is crushed by the expanding megagametophyte (Figs. 1, 5). After these cells have been crushed, the remaining intact chalazal nucellus develops into the chalazal proliferating tissue (Figs. 1, 5). The changes which accompany the breakdown of the crushed nucellar cells and the structure and fate of the antipodals will be described here.

In a section through the chalazal nucellus of an ovule containing an immature megagametophyte (Fig. 1), the cells to be crushed by the growing embryo sac can be distinguished by their thick cell walls. The cytoplasm of these cells shows progressive stages of deterioration. The most distal (with respect to the embryo sac) cells in this group, and the last to break down, have cytoplasm characterized by a well developed ER, and large numbers of mitochondria and dictyosomes (Fig. 2). The irregular appearance of the inner surface of the thickened cell wall (Fig. 2) suggests the possible incorporation of the products of dictyosome vesicles (Mollenhauer & Morré, 1966).
Plastids, which are present in smaller numbers than mitochondria, have few internal lamellae and seldom contain starch. They are usually immediately adjacent to the nucleus. The nucleus is spherical, and contains a single nucleolus and scattered masses of chromatin (Fig. 1). Lipid droplets and spherical organelles (0.3–0.5 μm) surrounded by a single membrane occur in small numbers (Fig. 2). The single-membrane-bound organelles appear to be closely associated with the membranes of the ER and will be referred to as microbodies (Frederick, Newcomb, Vigil & Wergin, 1968).

Signs of progressive cytoplasmic deterioration are seen in the cells which are closer to the embryo sac. The first changes noted include the fragmentation of the ER and the disappearance of the dictyosomes (Fig. 3). Plastids are sharply reduced in number and are frequently seen enclosed, with fragments of the ER, within a double membrane-bound structure which resembles an autophagic vacuole (de Duve & Wattiaux, 1966) (Fig. 3). The appearance and number of mitochondria remain about the same. Lipid disappears but the number of microbodies per cell increases (Fig. 3). As breakdown proceeds the amount of ER is reduced (Fig. 4) and the nuclear and cell membranes are destroyed (Figs. 3, 4). As these cells are crushed by the growing megagametophyte the remaining cytoplasmic contents are apparently solubilized and absorbed by the embryo sac (Figs. 5, 6). Finger-like extensions of the chalazal embryo sac wall (Figs. 6, 7) which project into the developing endosperm increase the absorptive surface area of the plasma membrane of the central cell in this area.

The 3 antipodals remain intact (Figs. 1, 5, 8) while the proximal portion of the nucellus is being crushed by the growing embryo sac. Only later do they become disorganized and break down (Fig. 6). Each antipodal contains a large nucleus with a single, dense nucleolus (Fig. 8). The antipodal cytoplasm is packed with dense ribosomes but contains relatively few organelles. A few mitochondria and cup-shaped plastids (Schulz & Jensen, 1968a, b) are present but little ER and virtually no recognizable dictyosomes. Vacuoles of varying size may be present (Fig. 8). Occasionally the wall does not completely form between 2 antipodals so that 2 nuclei may appear within the same cell (Fig. 1). There are many plasmodesmata in the cell walls which separate the antipodals from each other and from the central cell cytoplasm (Fig. 8). Infrequently, plasmodesmata are seen in the walls which separate the antipodals from the chalazal nucellus (Fig. 9).

The cells of the nucellus are not cemented to the inner integument cells by a common middle lamella (Figs. 1, 5). Where they border each other, the outer wall surfaces of both cell types are delimited by an electron-dense line (Fig. 4) which may represent a thin cuticle-like substance. This separation allows the nucellar cells to be crushed without causing disruption to the adjacent integument cells (Fig. 5). In section the nucellus is often seen pulled away from the inner integument (Figs. 1, 5) as a result of shrinkage during tissue preparation. The walls of nucellar cells which border the megagametophyte appear to be fused with the embryo sac wall (Figs. 1, 5, 8).

All that remains of the crushed portion of the chalazal nucellus and the antipodals is a mass of PAS-positive cell wall material (Fig. 10) which lies adjacent to the residual nucellus. The cells of this residual intact nucellus will enlarge to form the chalazal proliferating tissue.
Development and breakdown of the chalazal proliferating tissue

From the first division of the zygote to the early globular stage of embryo development (Schulz & Jensen, 1968b, c) the cells of the chalazal proliferating tissue enlarge considerably to form a mound-shaped mass of tissue which protrudes into the chalazal end of the embryo sac (Figs. 6, 10, 11). During this period of growth the cells are densely cytoplasmic and stain for protein (Fig. 10) and nucleic acids (Fig. 11). The nuclei are large, contain one or more nucleoli and scattered masses of chromatin (Fig. 6). The rapid incorporation of nucleic acid precursors during this period of cell enlargement (Pollock & Jensen, 1967) suggests that these cells are in a polyploid condition. Plastids are found close to the nuclear membrane (Fig. 14); they have a few single internal lamellae and may contain starch. Mitochondria, containing short, vesiculate cristae, are present in greater numbers than plastids and have a random distribution in the cell. There is a moderate amount of ER which appears as single cisternae. Polyribosomes are plentiful and occur free or attached to the ER. Dictyosomes and lipid droplets are present and several small vacuoles are scattered throughout the cells. A few microbodies are seen, but there are no apparent multivesicular bodies present in the cells at this time (Fig. 14).

The chalazal proliferating cells reach their maximum size (about 50 × 20 μm) and begin to break down when the embryo is in the globular stage of development. Cell degeneration proceeds in a wave pattern beginning with the cells which are closest to the embryo sac and continuing until all have broken down. Shortly before cell disorganization large amounts of ER, stacked in groups of 3 to 8 parallel cisternae, appear in the cytoplasm (Fig. 15). Chains of polyribosomes can be seen attached to the membranes of the ER (Fig. 16). There is an apparent increase in the number of dictyosomes per cell (Fig. 17), and large vesicles, probably produced by the dictyosomes' cisternae, appear to be fusing with the cell membrane. The cell wall increases in thickness and shows irregularities in its surface contours which suggests the possible incorporation of the contents of dictyosome vesicles (Fig. 17). Microtubules are also seen in association with the cell wall (Fig. 18). Small (0.3-μm) multivesicular bodies, which contain 4-12 inner vesicles per thin section, appear for the first time (Figs. 15, 17). The nucleus becomes lobed (Fig. 20) but plastids (Fig. 17) and mitochondria (Fig. 20) remain unchanged in appearance.

As the cells begin to break down, electron density increases and plastids, which may still contain starch, aggregate in localized areas (Fig. 19). The position of this plastid polarization varies from cell to cell and does not appear to follow a pattern. Soon the ER fragments and the dictyosomes disappear (Fig. 20). Plastids, some mitochondria and pieces of ER are seen encircled by the membranes of what appear to be autophagic vacuoles (Fig. 21). There is an increase in the frequency of microbodies (Fig. 20) and multivesicular bodies (Fig. 22) per cell. Multivesicular bodies also increase in size (0.8 μm) and may show up to 50 internal vesicles per thin section. The cells become filled with many small vacuoles (Figs. 19, 22). Ribosomes appear to be attached to the membranes of some vacuoles (Fig. 22) which may develop from the dilation of pieces of rough ER. Concurrent with these changes, the cells stain more
intensely with aniline blue black (Fig. 12) and azure B (Fig. 13). This increased staining reaction appears to result from a combined increase in ribosome density (Fig. 23) and changes in the ground matrix which render it more electron-dense (Figs. 22–24).

Finally, plastids, starch, and lipid disappear and large vacuoles develop in some of the cells (Figs. 12, 13). The nucleus becomes disorganized and the cell membrane breaks down (Fig. 23). The end wall, which becomes thin by the apparent loss of fibrillar material from the surface of the wall (Fig. 23), ruptures. This allows the contents of the chalazal proliferating cells to mix with the endosperm (Fig. 24). These contents include intact mitochondria, ribosomes, and pieces of degenerated cytoplasm. The pieces of degenerated cytoplasm are surrounded by a membrane (Fig. 24) and appear to be the partially destroyed contents of autophagic vacuoles.

Large endosperm vacuoles in the immediate vicinity of the ruptured chalazal proliferating cells are filled with vesicles and what appears to be cytoplasmic debris (Fig. 24). Vacuoles of this type are not seen in other parts of the endosperm and may represent a possible mechanism for the destruction of material released from the chalazal proliferating cells.

**DISCUSSION**

The present report confirms the observations of Guignard (1902), Henry (1958), and Pollock & Jensen (1967) that the chalazal proliferating tissue of _Capsella_ develops from a group of chalazal nucellar cells and does not arise from a proliferation of the antipodals. The 3 antipodals, which degenerate shortly after fertilization, are easily distinguishable from the chalazal nucellus. Each antipodal has a large nucleus surrounded by a thin rim of cytoplasm which contains few organelles. Where they border each other and the central cell the antipodals are surrounded by a thin cell wall which is perforated by plasmodesmata. The presence of plasmodesmata in the walls separating the cells of the megagametophyte from each other and the absence of plasmodesmata in the walls separating the megagametophyte from the sporophyte generation is of general occurrence (Diboll, 1968; Diboll & Larsen, 1966; Schulz & Jensen, 1968) and may reflect a physiological interdependence among the cells of the megagametophyte. The presence of some plasmodesmata in the thicker cell walls which separate the antipodals of _Capsella_ from the chalazal nucellus (the fused megagametophyte-nucellus wall) is an exception to this observation which cannot be explained at this time.

The position of the antipodals and their persistence during the crushing of a portion of the chalazal nucellus by the expanding megagametophyte raises the question of the function of the antipodals and their role, if any, in the destruction of the nucellus. In grasses (Brink & Cooper, 1944) the antipodals proliferate to high numbers and emerge as active cells which metabolize nutrients to feed the developing endosperm. In maize, which has about 20 antipodals, these cells have a well developed ER and large numbers of active dictyosomes and mitochondria which are characteristics of cells engaged in high rates of synthetic activity (Diboll, 1968). Furthermore, in maize,
the presence of papillate extensions of the inner face of the antipodal wall adjacent to
the nucellus and of the outer face of the antipodal wall adjacent to the central cell
(which increases the absorptive surface area of the plasm membrane in these areas) is
interpreted as indicating a possible course of metabolite flow from nucellus to anti-
podal to central cell. Wall projections of this type have been observed in many plant
cells which are known to be actively engaged in absorption and secretion (Gunning &
Pate, 1969). Diboll (1968) has suggested that, in maize, the antipodals may have a
nutritive function similar to that of the synergids (Jensen, 1965a; Schulz & Jensen,
1968a).

In *Capsella* the presence of a few mitochondria and plastids, sparse ER and the ab-
sence of dictyosomes in the antipodal cytoplasm strongly suggest that these cells are
not highly synthetic. Therefore, it does not seem likely that the antipodals have a
specific nutritive function in *Capsella* or that they synthesize substances, e.g. enzymes,
for export and use in the destruction of the nucellus. The presence of wall projections
on the chalazal embryo sac wall outside the antipodals suggests that metabolites from
the crushed nucellus may be absorbed directly by the central cell without first being
metabolized by the antipodal cytoplasm. On the basis of the present observations it is
difficult to assign a specific functional role to the antipodals of *Capsella*.

The chalazal proliferating tissue is located in the path between the termination of
funicular vascular strand and the chalazal end of the embryo sac. The position of these
cells at the terminus of the vascular supply may, in part, account for their great in-
crease in size. As the chalazal proliferating cells approach their maximum size they
appear to enter into a period of increased synthetic activity characterized by a prolifera-
tion of ER and an increase in the number of active dictyosomes per cell. Nucleic acid
precursors are actively incorporated into the cells at this time (Pollock & Jensen, 1967).
It is appealing to speculate that the chalazal proliferating cells may be receiving
nutrients from the vascular supply which they process and store for the use of the
developing embryo and endosperm. The chalazal embryo sac wall projections could
serve the dual function of facilitating the absorption of the degraded cytoplasm of the
crushed nucellus and the absorption of substances metabolized by the chalazal pro-
liferating cells. In *Capsella* similar wall projections occur in the basal cell (Schulz &
Jensen, 1969) and the synergids (Schulz & Jensen, 1968a), which are cells considered
to be active in the absorption of metabolites from the nutrient-rich integument.

These observations strongly support the suggestion that the chalazal proliferating
cells may perform a function similar to the nutritive function of the antipodals of grass
and maize (Brink & Cooper, 1944; Diboll, 1968), and the synergids of cotton (Jensen,
1965a), and *Capsella* (Schulz & Jensen, 1968a). These cells may also represent a form
of hypostase (Maheshwari, 1950) which is a group of chalazal nucellar cells which has
been assigned the variable functions of supplying growth substance, regulating the
water economy of the embryo sac, and acting as a barrier to prevent further chalazal
growth of the megagametophyte. Elucidation of the precise nature of the synthetic
activity of the chalazal proliferating cells and the direction of metabolite flow to and
from these cells must await further study.

A study of the changes which accompany cell disorganization in the chalazal
proliferating tissue and in the portion of the chalazal nucellus which is crushed by the megagametophyte reveals some interesting comparisons. In both cases cell breakdown is immediately preceded by a period of apparent increased metabolic activity accompanied by a thickening of the cell wall. It is difficult to suggest an explanation for this wall thickening because the fate of the cell wall is different in each case. The walls of the crushed portion of the chalazal nucellus remain intact long after the complete resorption of the cytoplasm of these cells and may never completely break down. In contrast, the walls of the chalazal proliferating cells become thin and rupture to release the cytoplasmic contents of these cells into the embryo sac. The thickening of the wall prior to cell disorganization also occurs in the persistent synergid of cotton (W. A. Jensen, unpublished observations).

In both the chalazal proliferating tissue and the crushed nucellus an increase in number of single-membrane-bound organelles parallels cytoplasmic disorganization. These organelles resemble in size and appearance the plant microbodies described by Frederick et al. (1968), Catalase (Vigil, 1969) and certain oxidases (Breidenbach, Kahn & Beevers, 1968) have been localized in similar plant organelles. The histochemical identification and significance of the increased number of these organelles in the degenerating tissues of Capsella is not known at this time.

The presence of large numbers of multivesicular bodies in disorganizing chalazal proliferating cells may be related to the fact that these organelles are considered to be a form of lysosome (Hruban & Rechcigl, 1969) and are involved in lytic processes in cells (Smith & Farquhar, 1966). Multivesicular bodies have also been described in the suspensor of Capsella (Schulz & Jensen, 1969) and the nucellus of cotton (Jensen, 1965a) which are structures destined to degenerate. The apparent absence of multivesicular bodies in the degenerating cytoplasm of the crushed nucellus cannot be explained.

The early breakdown of the plastids during the disorganization of both the chalazal proliferating tissue and the crushed nucellus may be related to a cessation of synthetic activity and storage of metabolites. In both tissues plastids are frequently seen enclosed within membrane-bound structures resembling autophagic vacuoles (Swift & Hruban, 1964). On the basis of these observations it seems probable that the plastids in these tissues are disposed of by being digested in autophagic vacuoles. Similar autophagic vacuoles, containing portions of sequestered cytoplasm and partially digested organelles, have been described in the degenerating suspensor cells of Capsella (Schulz & Jensen, 1969). At the present time no explanation can be offered for the strong polarization of the plastids prior to their disappearance in the chalazal proliferating cells.

Although some mitochondria may also be trapped in autophagic vacuoles, many mitochondria persist until the final stages of cell disorganization in both the chalazal proliferating tissue and the crushed nucellus. These persisting mitochondria could provide an energy source for catabolic reactions.

Some interesting parallels can be drawn between the chalazal proliferating cells and the persistent synergid in Capsella (Schulz & Jensen, 1968a). In both instances the cells appear to be synthetically active prior to breakdown. There is evidence that both
cell types may be engaged in metabolizing and storing nutrients from surrounding ovular tissue for the use of the developing embryo sac. Some similarities are also noted in the manner of disorganization of the chalazal proliferating cells and the persistent synergid. In both cases, after the nucleus becomes disorganized, the end wall ruptures and portions of intact cytoplasm mix with the cytoplasm of the central cell. This manner of disorganization stands in contrast to the destruction of the chalazal nucellar cells which are crushed by the growing megagametophyte. In the latter, the cell walls remain intact and the atrophied cytoplasm is apparently solubilized before being absorbed by the growing embryo sac. These observations tend to support the conclusion that different mechanisms of cell disorganization may be operative within the same organism.

This research was supported by NSF grant GB 3460.

REFERENCES


(Received 26 August 1969—Revised 28 July 1970)
Fig. 1. Chalazal nucellus, including future chalazal proliferating tissue (cp), of an ovule containing an immature megagametophyte. That portion of the chalazal nucellar cells (cn) which will be crushed by the growing embryo sac (es) has thick walls and shows progressive stages of cytoplasmic deterioration. Two of the 3 antipodal nuclei (arrow) are within the same cell. Shrinkage during tissue preparation causes the nucellus to pull away from the inner integument (in). KMnO₄, × 3000.
Chalazal proliferating tissue of Capsella
Fig. 2. Cytoplasm of chalazal nucellar cell just before breakdown. A well developed ER (er), active dictyosomes (d), mitochondria (m), plastids (p), microbodies (mb), and lipid droplets (l) are present. Thickened, irregular cell walls (cw) may be incorporating products of the dictyosomes' vesicles (arrows). KMnO₄, × 21725.

Fig. 3. Chalazal nucellar cell showing early signs of cytoplasmic disorganization. Dictyosomes are absent, ER is fragmented, plastids (p) are frequently seen surrounded by membranes resembling autophagic vacuoles. The frequency of microbodies (mb) increases. Mitochondria (m) remain the same in appearance and distribution. KMnO₄, × 27650.

Fig. 4. Final stage of cytoplasmic deterioration of chalazal nucellar cells. Note the reduced amount of ER, the absence of the cell membrane (arrow), the disorganization of the nucleus (n) and the persistence of the mitochondria (m). An electron-dense cuticle-like substance (double arrows) covers the outer cell wall surfaces of the nucellus (cn) and inner integument (in) where they border each other. KMnO₄, × 16600.
Chalazal proliferating tissue of Capsella
Fig. 5. Chalazal end of embryo sac (es) at egg stage showing intact antipodals (an), remains of chalazal nucellar cells (en) crushed by the growing megagametophyte and the chalazal proliferating tissue (cp). GA-OsO₄, × 2840.
Chalazal proliferating tissue of Capsella
Fig. 6. Section through enlarged chalazal proliferating cells (cp) of an ovule containing an octant embryo and well developed nuclear endosperm (en). Compare the size of the chalazal proliferating cells with those in Fig. 5 at the same magnification. Notice the remains of a degenerated antipodal (an) and that portion of the chalazal nucellus (en) crushed by the megagametophyte. Wall projections (wp) occur on the chalazal embryo sac wall. GA-OsO₄, ×2840.

Fig. 7. Enlarged view of projections (wp) of chalazal embryo sac wall which extend into endosperm (en) and increase the absorptive surface area of the plasma membrane (arrow) of the central cell. (cp, chalazal proliferating cell.) GA-OsO₄, ×23 100.
Chalazal proliferating tissue of Capsella
Fig. 8. Antipodal (an), and part of a second one, containing a large, dense nucleus (n) and nucleolus (nu) and very few mitochondria (m) and cup-shaped plastids (p). Plasmodesmata (arrows) are seen in walls separating antipodals from each other and from the cytoplasm of the embryo sac (es) (central cell). The walls of nucellar cells (cn) which border the megagametophyte appear to be fused with the embryo sac wall. (cn, chalazal nucellus; v, vacuole.) GA-OsO₄, × 23000.

Fig. 9. Some plasmodesmata (pd) occur in the walls (cw) which separate the antipodals (an) from the adjacent chalazal nucellus (cn). (es, embryo sac.) GA-OsO₄, × 14 500.
Chalazal proliferating tissue of Capsella
Fig. 10. Longitudinal section through chalazal proliferating tissue (cp) at egg stage showing adjacent mass of PAS-positive cell wall remains (arrows) of crushed antipodals and nucellar cells. Endosperm nucleus (en) and cytoplasm of the chalazal proliferating cells stain for protein. (es, embryo sac.) PAS and aniline blue black, × 1360.

Fig. 11. Chalazal proliferating tissue of an ovule containing an early globular embryo showing staining reaction for nucleic acids. The intensity of the stain increases as cells begin to break down. (es, embryo sac.) Azure B, × 530.

Fig. 12. Chalazal proliferating tissue of an ovule containing a globular embryo showing increased staining of cells in progressive stages of breakdown. Note multiple nucleoli and large vacuoles in some cells. (en, endosperm; es, embryo sac.) Aniline blue black, × 530.

Fig. 13. Increased staining and increased vacuolation are characteristic of progressive deterioration of chalazal proliferating cells of an ovule containing a heart-shaped embryo. (en, endosperm; es, embryo sac.) Azure B, × 530.
Fig. 14. Cytoplasm of chalazal proliferating cells during early growth period (zygote stage of development) showing plastids (p) containing starch (s), mitochondria (m), ER (er), dictyosomes (d), microbodies (mb), vacuoles (v), and lipid droplets (l). (n, nucleus.) GA-OsO₄, $\times 23,800$. 
Chalazal proliferating tissue of Capsella
Figs. 15–18. Cytoplasm of mature chalazal proliferating cells.

Fig. 15. Note parallel grouping of ER (er) cisternae, dictyosome (d) activity and multivesicular bodies (mvb). (l, lipid; v, vacuole.) GA-OsO₄, × 26 000.

Fig. 16. Chains of polyribosomes are attached to membranes of the ER seen here in surface view. GA-OsO₄, × 46 000.

Fig. 17. Dictyosome cisternae (d) produce large vesicles (arrows) which appear to be fusing (double arrow) with the plasma membrane. Cell walls (ctc) are irregular in appearance. (mvb, multivesicular body; p, plastid; v, vacuole.) GA-OsO₄, × 26 000.

Fig. 18. Microtubules (arrow) are associated with the cell wall (ctc). GA-OsO₄, × 46 000.

Figs. 19–24. Cytoplasmic changes which accompany the breakdown of the chalazal proliferating cells.

Fig. 19. Portions of chalazal proliferating cells in different stages of breakdown. Note the aggregation of starch-containing (s) plastids (p) and the increase in electron density. The cytoplasm of dense cell at centre right is filled with small vacuoles (v). GA-OsO₄, × 9400.

Fig. 20. The ER (er) disperses and fragments and the frequency of microbodies (mb) increases. Note the lobed nucleus (n). (m, mitochondrion.) KMnO₄, × 16 500.
Fig. 21. Plastids (p), mitochondria (m), and ER (er) in membrane-bound structures resembling autophagic vacuoles. KMnO₄, × 26000.

Fig. 22. Multivesicular bodies (mvb) increase in number and size and may contain up to 50 internal vesicles per thin section. Ribosomes (arrow) are attached to the membrane of some vacuoles (v). (l, lipid.) GA-OsO₄, × 50000.

Fig. 23. The cell membrane disappears (arrows) and the cell wall (cw) begins to break down. Note density of ribosomes in chalazal proliferating cell (cp). (en, endosperm.) GA-OsO₄, × 46200.

Fig. 24. Rupture (arrow) in end wall (ecw) of chalazal proliferating cell (cp) and mixing of intact mitochondria (m) and ribosomes with endosperm (en). Portions of degenerated cytoplasm (dc) within the chalazal proliferating cells appear to be surrounded by a membrane (double arrows). A large endosperm vacuole (upper left) near rupture is filled with cytoplasmic debris. GA-OsO₄, × 23100.
Chalazal proliferating tissue of Capsella