Proc. Indian natn. Sci. Acad. **B54** No. 1 pp. 93–110 (1988)

Ultrastructure and Histochemistry of Angiosperm Embryo Sac—An Overview

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(Received 5 October 1987)

The angiosperm female gametophyte is a specialized functional unit derived after the process of megasporogenesis and megagametogenesis. A tetrad is produced after process of megasporogenesis. In the megaspore tetrad usually all the three micropylar cells degenerate and the chalazal one becomes functional. In the latter the number of plastids, mitochondria, dictyosomes and ribosomes increase. The degenerating megaspores furnish metabolites to the functional megaspore which divides mitotically leading to the development of the female gametophyte.

The ultrastructural and histochemical studies of mature embryo sac indicate many specialized functions performed by the various component cells. Among these the synergids appear to be most dynamic and complex. They perform the function of absorption on account of massive wall-ingrowths of the filiform apparatus. They are also involved in the active transport and storage of nutrients due to high concentration of mitochondria near the filiform apparatus, and presence of dictyosomes, ER, lipids, starch grains, high concentration of proteins and RNA.

The cytoplasmic organelles in the egg cell are less abundant and it is presumed that it is metabolically less active than the other cells of the mature embryo sac. The scarcity of ER and dictyosomes indicate a reduced capacity for synthetic metabolism. The central cell is the largest cell of the mature embryo sac and appears to be engaged in intense metabolic activity with its extensive ER, mitochondria, dictyosomes, microbodies and other cell organelles. Storage reserves of the central cell are used for the development of young endosperm. The presence of well-developed, wall-projections suggests that the central cell absorbs large quantities of nutrients from the surrounding tissue and helps in the nutrition of the egg through the wall less areas between the central cell and the egg.

In a few taxa precocious degeneration of antipodes occurs. This aspect is least understood. When persistent the antipodal cells are the most variable cells of the megagametophyte. In several plants their nuclei show dense staining for DNA, endomitosis and polyteny. They are also rich in total proteins, RNA and histones. Ultrastructural studies reveal that the antipodal cells are metabolically very active being rich in ER, ribosomes, mitochondria, dictyosomes and plastids.

Key Words: Angiosperm embryo sac, Ultrastructure, Histochemistry

Introduction

During the last two decades, much information has been gathered concerning the female gametophyte in angiosperms using histochemical and ultrastructural techniques. The female gametophyte/mature embryo sac is organised after megasporogenesis and megagametogenesis. This overview briefly collates the data dealing with the fine structure and histochemistry of (i) archesporial cell and megasporocyte, (ii) callose deposition during megasporogenesis, (iii) cytoplasmic and nuclear changes during megasporogenesis, (iv) functional megaspore, (v) megagametogenesis, and (vi) cells of the organized embryo sac.

Archesporial Cell and Megasporocyte

The archesporial cell either functions directly as the megaspore mother cell or divides into an outer parietal and an inner primary sporogenous cell. The latter gives rise to the megaspore mother cell. In *Nigella*

damascena the hypodermal archesporial cell divides to form the primary parietal cell and primary sporogenous cell. Occasionally the nucellar protoderm divides periclinally and adds to the parietal tissue (Vijayaraghavan & Marwah 1969).

The archesporial cell in different cultivars of *Dendrobium* has extensive tubular ER, numerous lipid microdroplets, mitochondria and plastids (Israel & Sagawa 1964). In *Lilium* this cell can be distinguished from the surrounding cells by its large size and pronounced nucleus (de Boer-de Jeu 1978). The nucleus has nucleolema consisting of ribonucleoprotein particles. The cytoplasm is rich in RER, mitochondria, plastids and ribosomes. Plasmodesmatal connections are present between the archesporial cell and the surrounding nucellar cells in the initial stages of archesporial cell development but later these connections are broken/disappear (Israel 1963). A large number of small vacuoles are also present (Tilton 1981a, figure 1A, B).

Megasporocyte

The cytoplasmic contents of the megasporocyte are at a lower ebb, during prophase I, in the megasporocyte than in the archesporium. This decrease may be due to the enlargement of the cell and/or due to the using up of these substances by the developing megaspore mother cell.

The megaspore mother cell shows polarized distribution of organelles in many of the investigated plants. In *Oenothera lamarckiana* (Jalouzot 1971), *Epilobium* (Bednara & Rodkiewicz 1974, Rodkiewicz & Bednara 1974) and in ten other taxa of Onagraceae (Rodkiewicz & Sniezko 1978) plastids are located at both the poles of the megasporocyte. Plastids containing starch grains are localized within the micropylar portion of the megasporocyte during early meiotic prophase and during prophase are present in close proximity to the nuclear envelope in *Epipactis* (figure 1D). Plastids are localized either in the perinuclear zone or at the chalazal end in *Zea mays* (Russell 1979), and concentrated in the perinuclear cytoplasm in *Dendrobium* (Israel & Sagawa 1965). Rough endoplasmic reticulum is reported to be abundant in the micropylar-half of the megaspore mother cell and lies parallel to its micropylar wall in *Zea mays*. Such polarized distribution of endoplasmic reticulum indicates the temporal relations of the future functional megaspore (Russell 1979). In *Helianthus annuus*, the megaspore mother cell cytoplasm contains short strands of ER. Mitochondria and relatively undifferentiated plastids are located around the nucleus (Newcomb 1973). In *Paphiopedilum spicerianum* (Corti & Cecchi 1970) and *Allium cepa* (de Boer-de Jeu 1978) the nucleus is present at the micropylar region of megasporocyte and organelles such as mitochondria, plastids, dictyosomes and ER are located at the chalazal end.

In *Lilium candidum* during the meiotic prophase of megasporocyte extensive parallel cytoplasmic membranes of ER gets transformed into concentric whorls which encloses portions of the cytoplasm containing mitochondria, osmiophillic, droplets and multimembrane cytoplasmic bodies (Rodkiewicz & Mikulska 1964a, b, 1965, 1966). Such structures are also reported in *Paphiopedilum spicerianum* and *Lillium longiflorum* (figure 1E).

Dickinson and Andrews (1977) emphasize that the encapsulated cytoplasm of the megasporocyte fulfils two roles: (i) it carries reserves necessary for the postmeiotic development from the diplophase to the haplophase environment, (ii) it allows protein synthesis to continue throughout meiosis I and II, a period when a major part of protein synthetic apparatus is absent. Towards the end of the first meiotic prophase, the external membranes of the multilayered bodies become vesiculated, resulting in the formation of small concentric bodies, cores with double membrane and numerous vesicles scattered in the cytoplasm.

In *Lilium* small vesicles and tubules containing electron dense material occupy the extracytoplasmic space between the cell wall and the plasmamembrane. These vesicles are called paramural bodies believed to be involved in the synthesis of cell wall (de Boer-de Jeu 1978). Wall ingrowths are present at early meiotic prophase in the chalazal wall of the megaspore mother cell in *Epipactis* (Bednara 1978).

No plasmodesmatal connections were observed in the cell wall of the megaspore mother cell of *Myosurus minimus* (Woodcock & Bell 1968). At premeiotic interphase in *Lilium*, *Allium* and *Impatiens* plasmodesmata are present on all sides of the megaspore mother cell. After the premeiotic interphase, however, they are localized only towards the chalazal region of the megaspore mother cell (de Boer-de Jeu 1978). No plasmodesmata are present between the megaspore mother cell and adjacent nucellar cells after metaphase I. In *Zea mays*, a few plasmodesmata are present exclusively in the chalazal megasporocyte walls, indicating the probable direction of the supply of nutrients.

Cytoplasmic and Nuclear Changes during Megasporogenesis

A cyclic development of plastids and mitochondria is reported to occur in *Lilium longiflorum* (Dickinson & Heslop-Harrison 1977, Dickinson & Potter 1978). There is also an apparent elimination and restoration of the ribosomal population in the cytoplasm. In *Lilium*, *Allium cepa* and *Impatiens walleriana* the number of ribosomes per cytoplasmic area decreases during premeiotic interphase and early meiotic prophase (de

Boer-de Jeu 1978). After metaphase I, the number of ribosomes per cytoplasmic area increases, due to a ribosome-flow caused by the disintegration of the nuclear envelope (Willemse & Linskens 1968) and by breakdown of nucleolus-like bodies in the cytoplasm (Williams et al. 1973).

Morphological changes in the mitochondria and the plastids occur during megasporogenesis in *Lilium*, *Allium* and *Impatiens* (de Boer-de Jeu 1978). The mitochondria in *Lilium* and *Impatiens* contain numerous cristae and are electron-transparent during leptotene. From the zygotene stage onwards they become small and possess an electron dense matrix. Condensed mitochondria occur during premeiotic prophase in *Allium*, leptotene and pachytene stages in *Lilium* and at diplotene stage in *Impatiens*. In *Lilium* the number of spherical and dumb-bell shaped plastids decrease during meiotic prophase but those that are cup-shaped increase. The number of thylakoids and plastoglobules also decrease and the matrix becomes more electron transparent due to the loss of ribosomes. In *Allium*, the thylakoids are always found in plastids during megasporogenesis but in *Lilium* and *Impatiens* the thylakoids are present only at premeiotic interphase and meiotic prophase. The inner membrane of nucleus shows sacculation during meiotic prophase in the above three taxa. Maximum sacculation of the inner nuclear membrane occurs in *Allium* and *Impatiens* at the diplotene stage and is caused by the loss of rigidity of the nuclear envelope.

Synaptinemal complexes are formed due to chromosomal pairing at zygotene as in *Lilium, Allium* and *Gasteria verrucosa* (de Boer-de Jeu 1978, Willemse & Franssen-Verheijen 1978, Willemse & Bednara 1979). Membrane-like particles are found in the nuclei at zygotene and at diplotene. In *Impatiens* and *Lilium* nucleolus-like bodies with condensed, granular, electrondense appearance are perceptible after metaphase I. In *Anemone pavonina* such nucleolus-like globules in the cytoplasm contain RNA and have been identified as "nucleoloids" (Ünal 1978). In *Ornithogalum caudatum* the nucleus of the functional megaspore is larger than those of the other three non-functional megaspores and has a large central nucleolus, one or more small nucleoli and fused chromatin around the periphery. The cytoplasmic organelles are initially, uniformly distributed in the cytoplasm but migrate later towards the micropylar region. In *Epilobium* numerous plastids with starch, mitochondria and dictyosomes are present in the functional megaspores (Bednara & Rodkiewicz 1974).

Out of these four megaspores produced invariably the chalazal one functions. Several exceptions to this generality are known to occur. In *Robinia pseudo-acacia* (Rambert 1969), *Gasteria verrucosa* (Willemse & Bednara 1979, Willemse & Kapil 1981b) and *Pongamia pinnata* (Seshavatharam & Subba Rao 1982) triads are produced after megasporogenesis. These arise as a result of non-synchronous division in the dyad cells. The chalazal dyad cell divides earlier so that a row of three cells is produced. In *Epilobium palustre* triads are produced due to failure of cytokinesis in the binucleate chalazal cell of the dyad. Low dictyosome activity in the zone of cell-plate formation is the raison d'être for this diversion (Bednara 1977). In *Tellima grandiflora* both monosporic (Polygonum) and bisporic (Allium) types of embryo sac development are reported (Ratnaparkhi 1973). In tetrasporic type of embryo sac development the dictyosomes and ER do not produce vesicles responsible for cell-plate formation (de Boer-de Jeu 1978).

The dyads that undergoes meiosis show an intense staining of the chromosomes for ascorbic acid (Shah & Pandey 1977) but a feeble staining for cell plate and spindle fibres. The degenerating micropylar megaspore stains deeply for ascorbic acid, whereas the functional chalazal megaspore stains only moderately. The upper degenerating dyad nucleus stains poorly with pyronin Y as compared to the chalazal one. Proteins, aminoacids and lipids are more concentrated in the chalazal functional megaspore than in the upper one (Willemse & Bednara 1979). There is a gradual increase in the concentration of RNA and proteins in the megaspores after the first and second meiotic divisions in *Argemone mexicana* (Bhandari et al. 1980). Non-functional megaspores are characterized by a very intense staining for all the metabolites (RNA, proteins and DNA). This may be due to rapid disintegration of all the organelles in these cells resulting in loss of specific sites of localization of metabolites. The nucleus of the degenerating megaspores is small, dense and stains intensely for proteins and RNA in *Ornithogalum*. Condensation of the nucleoplasm may be responsible for the increased affinity for the stains.

Callose Deposition During Megasporogenesis

Callose-a (β 1-3 polyglucan) in plant tissue is detected by its property to fluoresce when coupled with a fluorescent dye. Callose was detected for the first time with the help of fluorescent microscope in *Orchis maculata*. Since then it has been noticed during megasporogenesis in 43 plants belonging to 14 families. However, callose is absent in *Lilium candidum, L. regale* and *Tulipa* sp., which shows tetrasporic type of embryo sac development (Rodkiewicz 1970). Schwab (1971) observed the presence of callose during megasporogenesis in *Diarrhena* and since then it has been reported to occur in *Agrostis interrupta, Festuca microstachys, Stipa elmeri* and *Zea mays* (Maze & Bohm 1974, 1977, Russell 1979).

The callose deposition in the megasporocyte wall occurs during early meiotic division and in the Polygonum type of embryo sac development it appears only in the chalazal pole of the megaspore mother cell. In the Oenothera type of development the callose is localized at the micropylar pole (Rodkiewicz 1967, 1968, 1970, Kuran 1972, Noher de Halac & Harte 1975). By meiotic metaphase, the megasporocyte wall is completely enveloped by callose and during dyad and tetrad stages it is also present in the transverse walls that separate the two cells. In *Oenothera muricata* (Rodkiewicz & Kuran 1971, Rodkiewicz et al. 1971) and *O. biennis* (Noher de Halac 1980, Noher de Halac & Harte 1975, 1977) callose is absent on the external cell walls but is present on the transverse walls in the megaspore tetrads. This is because of 'Renner's effect'—a condition in which the functional megaspore can develop either from the micropylar cell or from any other cell of the tetrad. In the Polygonum type of embryo sac development callose disappears from the sides of the functional, chalazal, megaspore and in Oenothera type reverse situation is observed. This sudden appearance and disappearance of callose is possible only due to a high polymerization and depolymerization ability of this polysaccharide.

The deposition of callose in the walls during megasporogenesis is a specific feature of several taxa. In *Epilobium palustre* and *Fuchsia* sp. the callose in cross wall is partially hydrolysed shortly after the formation of a tetrad resulting in sites with little callose that simulate the sieve-plates. The nutrient supply from the degenerating megaspores to the functional megaspore may thus be possible from these sites. Similarly in *Orchis, Epipactis* and *Cephalanthera* there are numerous small areas devoid of callose in the chalazal wall of the megasporocyte (Rodkiewicz & Bednara 1976). This chalazal wall may be specialized for nutrient translocation among meiocyte, functional megaspore and somatic cells. It is even conjectured that the callose wall functions as a molecular filter and controls the development of the active megaspore (Heslop-Harrison 1964). However the general absence of callose in megaspore mother cell that forms tetrasporic type of embryo sac development (Rodkiewicz 1968, 1970) and its apparent absence in *Ornithogalum caudatum* (Tilton 1981a) suggest that callose deposition is not a prerequisite for megaspore formation and meiosis.

Functional Megaspore

The functional megaspore is the progenitor of the embryo sac. In *Myosurus minimus* the maturing functional megaspore contains vesicles that contain varying amounts of amorphous and opaque materials. During vacuolation of the megaspore this material disappears. In *Paphiopedilum spicerianum* the cytoplasmic organelles are preferentially distributed in the micropylar pole of the functional dyad cell but most of the vacuoles are present on chalazal pole (Corte & Cecchi 1970). In *Aquilegia vulgaris* the functional megaspore which has a thick-wall and little plasmodesmatal connections (Rifot 1971) contains a large number of cytoplasmic organelles. In *Helianthus annuus* the functional megaspore increases in volume followed by an increase in the number of free-ribosomes (figure 2A). The degenerating micropylar megaspores appear to provide nourishment to the functional megaspore (Newcomb 1973).

The functional megaspore enlarges, that is followed by the formation of vacuoles at micropylar and chalazal poles in *Pelargonium* (Tsai et al. 1973), by one large vacuole at the chalazal pole in *Stellaria media* (Kudlicka et al. 1981, figure 2B), possession of more plastids than in the degenerating megaspores in *Epilobium* (Bednara & Rodkiewicz 1974). In the enlarging megaspore of *Gasteria verrucosa,* the number of plastids, mitochondria, lipid granules, dictyosomes and ribosomes increases. Before mitosis the plastids are filled with starch which is utilized during gametogenesis.

Russell (1979) in *Zea mays* noted that soon after the formation of the functional megaspore vacuolization occur and cytoplasmic polarity is inconspicuous except for perinuclear distribution of plastids. Chromatin is evenly distributed in the nucleus and nucleolus has a well-developed nucleolar organizer region. The concentration of ribosomes and RER also increases and plasmodesmata occur infrequently in the chalazal regions (figure 2C). An electron dense layer formed on the inner surface of the wall persists during the formation of the functional megaspore. In *Ornithogalum caudatum* the nucleus of the functional megaspore is larger than of the degenerating megaspores and has a large, central nucleolus and many small nucleoli (figure 2D, E).

Active synthesis of RNA and protein has been reported in *Stellaria media* (Pritchard 1964), *Vanda* (Alvarez & Sagawa 1965), *Farsetia hamiltonii, Eruca sativa* (Prasad 1977) and *Argemone mexicana* (Bhandari et al. 1980) and that of lipid granules in *Paspalum longifolium* (Yu & Chao 1979). In *P. longifolium* cytoplasm is initially diffuse and stains lightly with Aniline-blue-black (ABB) but when the functioning megaspore elongates the cytoplasm undergoes vacuolation and condensation resulting in a strong positive reaction. Bennet et al. (1973) observed in wheat, rye and barley that the 4 C level of DNA in megaspore mother cell during first prophase was reduced to 2 C in dyad and to 1 C level in each of the four megaspores.

Megagametogenesis

A large number of cytoplasmic changes occur during megagametogenesis. In *Myosurus minimus* the ribosomes are uniformly distributed at the beginning of the 2-nucleate stage but occur in groups at later stages (Woodcock & Bell 1968). At the 4-nucleate embryo sac stage a gradual increase in the amount of RER, dictyosomes, and lipid droplets is reported in *Helianthus annuus* (Newcomb 1973). In *Gasteria verrucosa* abundant polysomes occur (Willemse & Franssen-Verheijen 1978). In *Zea mays* ribosomes, mitochondria and plastids are abundant at the 2-nucleate stage (Russell 1979). The 4-nucleate megagametophyte possesses a lytic complex produced due to dilated cisternae of ER. Cristae are rarely observed in the mitochondria but an electron-dense material is present that may represent the remnants of cristae appressed to the periphery (Russell 1979).

In *Nicotiana* the micropylar nucleus has DNA in the form of small clots and thin chromatin threads, whereas the chalazal nucleus shows dense chromatic threads that are homogenously distributed.

RNA concentration is high in the micropylar and chalazal poles of the 8-nucleate gametophyte while the central cell is weakly pyroninophilic. The antipodal nuclei are rich in DNA whereas the polar nuclei are poor. There is a reduction in DNA content during gametogenesis caused by stretching of DNA molecule and stable blockage in the protein, resulting in the fall of metabolic processes that might be prerequisite for embryogenesis (Bannikova 1971).

Organized Embryo Sac

In the majority of angiosperms the mature embryo sac is a seven-celled structure. The egg apparatus at the micropylar end of the embryo sac constitutes two synergids and an egg cell, the 3 antipodal cells are located at the chalazal end and in the middle is the central cell.

The outermost wall of the embryo sac lacks plasmodesmatal connections. In *Jasione montana* (Berger & Erdelska 1973) and in *Proboscidea loiusianica* (Mogensen 1978a, b), the wall of the mature embryo sac is cutinized. In *Stipa elmeri* the wall of the embryo sac consists of three portions. The innermost portion is the megagametophyte wall, middle is the remnants of the megaspore wall or remains of nucellar cells that are crushed during megagametophyte development and the outermost is the wall of nucellar cells (Maze & Lin 1975). In *Iberis amara* and *Alyssum maritimum* the embryo sac wall is PAS positive and shows wall ingrowths which are more prominent at the chalazal end. In *A. maritimum* this wall is associated with numerous cisternae of SER at the micropylar end (Prabhakar & Vijayaraghavan 1983).

Egg Cell

The egg cell is situated at micropylar end of the mature embryo sac and is partially surrounded by a wall which is thickest towards the micropylar end. No wall is seen towards the chalazal end in *Torenia fournieri* (van der Pluijm 1964), *Gossypium* (Jensen 1965a, b), *Linum usitatissimum* (Vazart 1969, Vazart & Vazart 1966), *Crepis tectorum* (Godineau 1969), *Petunia* (Van Went 1970b), *Quercus gambelii* (Mogensen 1972), *Helianthus annuus* (Newcomb 1973), *Stipa elmeri* (Maze & Lin 1975), *Proboscidea lousianica* (Mogensen 1978a) and *Nicotiana tabacum* (Mogensen & Suthar 1979). A little wall material is present at the chalazal end in *Capsella bursa-pastoris* (Schulz & Jensen 1968b), *Epidendrum scultella* (Cocucci & Jensen 1969a), *Plumbago zeylanica* (Cass & Karas 1974) and *Agave parryi* (Tilton & Mogensen 1979). A complete wall around the egg cell is reported in *Ornithogalum caudatum* (Tilton 1981b). The egg wall contains plasmodesmata where it borders the synergids and the central cell.

The egg is a polarized cell with the nucleus and much of the cytoplasm aggregating in the chalazal part, leaving a large vacuole towards the micropylar part. [*Zea mays* (Diboll & Larson 1966), *Crepis tectorum* (Godineau 1971), *Capsella bursa-pastoris* (Schulz & Jensen 1968b), *Petunia hybrida* (Van Went 1970a), *Quercus gambelii* (Mogensen 1972), *Helianthus annus* (Newcomb 1973), *Nicotiana tabacum* (Mogensen & Suthar 1979) and *Ornithogalum caudatum* (Tilton 1981b)]

In *Gossypium* the cytoplasm is confined to the basal region and the large vacuole occupies the micropylar end. A large single vacuole is usually present in the cytoplasm of the egg cell but in *Zea mays* (Diboll & Larson 1966), *Epidendrum scutella* (Cocucci & Jensen 1969a), *Capsella bursa-pastoris* (Schulz & Jensen 1968b) and *Festuca* (Maze & Bohm 1977) many small vacuoles are visible. A few plastids are present in the egg cytoplasm of several plants. The plastids in *Gossypium* also contain some lamellae, phytoferritin and ribosome-like particles which are approximately 100 A° in diameter.

The number of mitochondria is high in the egg cytoplasm of *Gossypium, Zea mays, Epidendrum scutella, Crepis tectorum, Helianthus annuus* and *Spinacia oleracea* (Wilms 1981a). However, this feature does not imply a high rate of respiration but a high potential for metabolic activity associated with post-fertilization phenomenon. In *Plantago lanceolata* the egg cell is characterized by the presence of cup-shaped mitochondria (Vannereau 1978) and in *Capsella bursa-pastoris* the mitochondrial matrix is less dense than that of the plastids with clear areas traversed by tiny, 22 A° fibrils.

Endoplasmic reticulum is abundant in the egg cytoplasm of *Gossypium* (Jensen 1965b) and *Quercus gambelii* (Mogensen 1972) but is less abundant in *Zea mays* (Diboll 1968), *Petunia* (van Went 1970b), *Capsella bursa-pastoris* (Schulz & Jensen 1968b), *Helianthus annuus* (Newcomb 1973), *Nicotiana tabacum* (Mogensen & Suthar 1979) and *Agave parryi* (Tilton & Mogensen 1979). In egg cell of *Gossypium* smooth tubular endoplasmic reticulum, RER and polysomes are present and dictyosomes are totally absent in *Epidendrum scutella* and *Nicotiana tabacum*.

An egg nucleus is large and contains a single large nucleolus in *Gossypium*, *Capsella bursa-pastoris*, *Linum catharticum*, *Stipa elmeri*, *Nicotiana tabacum*, *Agave parryi*, *Ornithogalum caudatum* and *Spinacia oleracea*. Micronucleoli occur in *Hordeum* (Cass & Jensen 1970). In *Nicotiana*, ergastic bodies resembling ribonucleoprotein are present near the egg nucleus (Dannikova 1971). Their composition and closeness to occurrence nor the nucleus indicates involvement in metabolic processes. In *S. oleracea*, myelin-like bodies are present for a short time in the karyoplasm of the interphase nuclei (Wilms 1981a, b).

In *Plumbago capensis* (Cass 1972; figure 3A) and in *Plumbago zeylanica* (Cass & Karas 1974) finger-like projections, resembling the filiform apparatus of the synergids, are present at the micropylar end of the egg cell. Interestingly, in both the taxa, synergids are absent during final organization of the embryo sac. The cytoplasm near the filiform apparatus is rich in microtubules, mitochondria, dictyosomes, ER, and polysomes. The microtubules are distributed near the FA and may participate in the positioning of the wall ingrowths and/or deposition of fibrillar materials in the projections. The ultrastructure of the egg cell of *Plumbago* indicates that this cell has a high metabolic activity and not only plays a role of nutrition but also helps in directional growth of the pollen tube through (egg's) the filiform apparatus.

A high concentration of RNA and proteins is noticed in the egg cell nucleolus and cytoplasm of *Stellaria media* (figure 3B). In *Vanda*, however, the cytoplasm (except at the periphery in contact with the synergids), exhibits a low profile of protein and RNA. In *Capsella bursa-pastoris* RNA and proteins are localized in the chalazal end. The egg cell shows the presence of amino acids in *Coix-lacryma jobi* (Bhatt & Shah 1973) and is rich in ascorbic acid in *Calanthe* and *Dendrobium* (Zinger & Poddubnaya Arnoldi 1966). In *Limnophyton obtusifolium* histones are localized in the chalazal region (Shah & Pandey 1977) and in *Nicotiana rustica* two strongly RNA-positive bodies are recorded (Sehgal & Gifford 1979). In mature embryo sac, the egg nucleus stains feebly with Feulgen stain and the reduction in DNA is due to increase in the size of the nucleus leading to the dilution of DNA content. Autoradiographic studies have shown a small amount of DNA in the egg nucleus of cotton (Fisher & Jensen 1969). In *Hordeum* the nuclei of the zygote and proembryo are at 4 C level (Bennett & Smith 1976). The egg cell wall is weakly PAS-positive and cytoplasm in many plants contains starch grains.

Synergids

The two synergids are structurally the complex cells (Vijayaraghvan & Bhat 1983; figure 3C). The cell wall around them is incomplete in *Torenia fournieri* (van der Pluijm 1964), *Gossypium* (Jensen 1965a), *Linum usitatissimum* (Vazart 1969, Vazart & Vazart 1966), Zea mays (Diboll & Larson 1966), *Quercus gambelii* (Mogensen 1972), *Helianthus annuus* (Newcomb 1973), *Stipa elmeri* (Maze & Lin 1975), *Proboscidea louisianica* (Mogensen 1978a), *Nicotiana tabacum* (Mogensen & Suthar 1979) and *Agave parryi* (Tilton & Mogensen 1979). In all these taxa a distinct wall is present around the micropylar one-third to one-half of the cell; it becomes thin towards the chalazal end and finally disappears. In contrast a distinct wall is present in the chalazal end in *Capsella bursa-pastoris* (Schulz & Jensen 1968a), *Epidendrum scutella* (Cocucci & Jensen 1969a), *Aqueligia formosa* (Vijayaraghavan et al. 1972) and *Ornithogalum caudatum* (Tilton 1981b). In *E. scutella* the chalazal wall disappears after fertilization. The wall of the synergids is PAS-positive in most of the angiosperm taxa but in *Jasione montana* heavy deposits of cutin occur in the micropylar portion (Berger & Erdelska 1973).

In *Capsella bursa-pastoris* and *Agave parryi* plasmodesmata are present among the cells of egg apparatus but not between cells of egg apparatus and the juxtaposed sporophytic cells. Plasmodesmata are absent in *Quercus gambelii* (Mogensen 1972, 1973). Dahlgren (1928) listed synergid-hooks in 52 species belonging to 22 families. In *Aquilegia formosa* (Vijayaraghavan et al. 1972) the hook cytoplasm is filled with numerous ribosomes; mitochondria and a large number of dictyosomes. Plastids and RER are also present. Vijayaraghavan et al. (1972) believe that the close association of these organelles in the cytoplasm of the hook of the synergid indicates that this region is also active (*see also* Mogensen & Suthar 1979, Sehgal & Gifford 1979, Tilton & Mogensen 1979).

The most prominent feature of almost all the synergids is the presence of filiform apparatus at the micropylar end that resembles transfer-cells (Gunning &

Pate 1969) involved in short distance transport. Filiform apparatus is absent in *Coronopus didymus, Brassica rapa, Farsetia hamiltonii, Lepidium sativum, Malochima africana* (Prasad 1975), *Jubaeopsis caffra* (Robertson 1976) and *Nicotiana rustica* (Sehgal & Gifford 1979). The ultrastructure of FA was first described in cotton by Jensen (1963) and its detailed ontogeny was reported in *Torenia fournieri* by van der Pluijm (1964).

The filiform apparatus of *Petunia* (van Went 1970b), *Helianthus annuus* (Newcomb 1973), *Proboscidea louisianica* (Mogensen 1978a) and *Nicotiana tabacum* (Mogensen & Suthar 1979) is only a thickening of the cell wall whereas in other taxa, it is an extension of synergid wall at the micropylar end of the cell (Schulz & Jensen 1968a, Jensen 1965a, Diboll & Larson 1966, Cass & Jensen 1970, Vazart 1971, Vijayaraghavan et al. 1972, Vannereau 1978, Yu & Chao 1979, Wilms 1981a, Bhandari & Sachdeva 1983). The plasma membrane follows the contours of the FA and greatly increases the surface area in these regions. In *Stipa elmeri,* FA is a highly convoluted extension of the micropylar wall and the cytoplasm is entrapped within its folds (Maze & Lin 1975). It consists of an outer translucent phase with loosely organized microfibrils in a matrix and an inner, electron-dense, region composed of tightly packed microfibrils. The FA in *Aquilegia formosa* consists of an electron-translucent central area surrounded by more electron dense protuberances of different shapes and sizes (figure 3D). In *Nicotiana tabacum* it is composed of fibrillar matrix filled with many osmiophilic bodies. The two halves of the FA lack a common wall in *Gossypium* but possess a common wall in other taxa.

The filiform apparatus stains intensely for insoluble polysaccharides as in a large number of plants examined. In *Zea mays* the polysaccharide nature of the PAS-stained materials is also proved by ultraviolet microscopy (Diboll 1967). The FA in *Proboscidea louisianica* and *Nicotiana tabacum* is, however, less PAS positive. FA stains for proteins in *Ornithogalum caudatum, Gossypium, Paspalum orbiculare, Aquilegia formosa, Proboscidea louisianica* and *Agave parryi,* but not in *Capsella bursa-pastoris* (Schulz & Jensen 1968a) and *Hordeum vulgare* (Cass & Jensen 1970). The FA in *Torenia fournieri* contain callose (Tiwari 1982), whereas, in *Petunia* cellulose (van Went 1970b).

Synergid Cytoplasm

The synergids are highly complex and active cells. The number and distribution of mitochondria in the synergids vary. They are abundant and concentrated near the FA in several taxa except in *Quercus gambelii* and *Agave parryi* where they accumulate at the chalazal end; are randomly distributed in *Ornithogalum caudatum* and uniformly dispressed in *Spinacia oleracea* (Wilms 1981b). In *Aquilegia formosa* mitochondrial cristae are short and ribosome-like particles fill the mitochondria (Vijayaraghavan et al. 1972).

Plastids are abundant in the cytoplasm of synergids in *Gossypium* (Jensen 1965a), *Helianthus annuus* (Newcomb 1973), *Zea mays* (Diboll & Larson 1966), *Stipa elmeri* (Maze & Lin 1975) and *Agave parryi* (Tilton 1975) but in *Epidendrum scutella* (Cocucci & Jensen 1969b), *Hordeum vulgare* (Cass & Jensen 1970), *Petunia* (van Went 1970b) and *Quercus gambelii* (Mogensen 1972), they are sparse. They are concentrated near the micropylar end in *Gossypium, Aquilegia formosa* (figure 3E), *Crepis tectorum* and *Ornithogalum caudatum.* In *Agave parryi* are localized near the chalazal end. Plastids contain starch and in *Epidendrum scutella* they store crystalline phytoferritin (Cocucci & Jensen 1969b).

Dictyosomes are abundant and consist of cisternae and vesicles being mostly concentrated near the FA in *Gossypium, Linum usitatissimum, Stipa elmeri* and *Ornithogalum caudatum* and are distributed throughout the cytoplasm in *Quercus gambelii* and *Aquilegia formosa.* In *Gossypium* dictyosomes show a morphological change along the length of the synergid. Dictyosomes associated with FA consist of 3 or 4 cisternae and a few vesicles. In the middle portion they contain a moderate number of large vesicles and 6 or 7 regularly arranged cisternae. The presence of a large number of dictyosomes in the peripheral region indicates their involvement in wall synthesis.

Endoplasmic reticulum is extensive in the synergid and oriented parallel to the long axis of the cell. Dilated ER, characteristic of secretory cells is abundant in *Aquilegia vulgaris* (Rifot 1971), *Conium maculatum* (Dumas 1978) and *Plantago lanceolata* (Vannereau 1978), stacked ER are seen in *Aquilegia formosa* (Vijayaraghavan et al. 1972).

The nucleus is well-developed, often large and contains a prominent nucleolus and micronucleoli. The nucleus is situated in the micropylar region of synergid in *Stipa elmeri,* and *Ornithogalum caudatum,* in the centre of the synergid cell in *Gossypium, Capsella bursa-pastoris,* and *Linum usitatissimum,* and in the chalazal part in *Proboscidea louisianica.* Nuclear pores are 900 A° in diameter. Ribosomes are associated with the outer nuclear membrane (Jensen 1965a).

The cytoplasm of the synergid is strongly polarized. Most of the cytoplasmic organelles are situated in the micropylar region whereas the chalazal region has either one large or many small vacuoles. In *Androcymbium* (Cave 1967) many small vacuoles are present. In *Linum usitatissimum, Aquilegia formosa* and *Ornithogalum caudatum* one large vacuole is present in the chalazal region whereas in *Gossypium,*

Proboscidea louisianica and *Nicotiana tabacum* a large chalazal vacuole along with many small vacuoles are present throughout the cytoplasm.

Synergids behave as haustoria in *Cortederia* (Philipson 1977, Philipson 1981). The basal part of the synergids elongates and grows out of the micropylar region. Ultrastructural studies reveal the presence of well-developed, transfer-cell wall invaginations, associated closely with the plasmamembrane and mitochondria. These wall projections occur over the whole inner surface of the haustorium (figure 3F), and aid in absorption and conduction of nutrients. Synergids are rich in total proteins and RNA in *Capsella bursa-pastoris* (Schulz & Jensen 1968a), *Vanda* (Alvarez & Sagawa 1965), *Nicotiana* (Bannikova 1971), *Argemone mexicana* (Bhandari et al. 1980) and *Ranunculus sceleratus* (Vijayaraghavan & Usha Bhat 1980). High amounts of peroxidase, cytochrome oxidase and ascorbic acid are present in the cytoplasm of *Zephyranthes rosea* (Malik & Vermani 1975) and abundant lipid granules surround the FA in *Paspalum longifolium* (Yu & Chao 1979). Insoluble polysaccharides are present in abundance in *Ranunculus tripartitus* (Patel & Cook, 1972), *Farsetia hamiltonii* and *Eruca sativa* (Prasad 1977) whereas in small quantities in *Lilium regale* (Georgieva 1965) and *Triticum* (Bennett et al. 1975).

In *Gossypium, Stellaria media* and *Quercus gambelii* the nucleus stains faintly with Azure B for DNA which may be due to 'dilution effect' brought about by the haploid condition and or due to the enlargement of the nuclei. In *Lilium regale* the mature synergid nuclei have thin chromatin filaments which give a positive reaction with Feulgen staining (Georgieva 1966). In *Allium cepa* one of the synergids becomes hypertrophied and stains densely with Azure B (Syamasundar & Panchaksharappa 1975).

Synergid Functions

(i) Synergids help in absorption, storage and transportation of nutrients from the surrounding tissue. The filiform apparatus, which is an extension of the micropylar wall of the synergids, greatly increases the surface area of the plasmamembrane and facilitates the absorption potentiality of the cell. The close association of mitochondria with the FA indicates that the energy required for active absorption of nutrients is supplied by them. Presence of a large number of plastids near the FA also indicates that the carbohydrates enter the synergids through FA and are also stored in plastids as starch. Endoplasmic reticulum functions as an internal transport system and is associated with the plasma membrane, vacuoles, mitochondria and plastids. Several plasmodesmatal connections present between synergids and central cell; synergid and egg help in the transportation of nutrients to the egg or central cell. In *Proboscidea louisianica* and *Nicotiana tabacum* the synergid wall forms evaginations towards the central cell cytoplasm and helps in the transportation of nutrients into the central cell. Presence of haustorial synergids as seen in *Cortaderia* suggests that the synergids are involved in absorption and transportation of nutrients.

(ii) Synergids help in the process of double fertilization. They secrete certain chemotropic substances which attract the pollen tubes towards them (van der Pluijm 1964). A PAS positive water soluble chemotropic substance is reported to be present in the micropylar region of *Paspalum orbiculare* (Chao 1971). According to Chao (1977) this substance is produced as a result of dissolution of cells of the integument by the activity of enzymes secreted by the synergids through the FA.

(iii) Synergids are also the site of pollen tube discharge and transmission of male gametes to egg and central cells in an orderly and regular manner. Ultrastructural studies on the synergids of *Gossypium*, *Torenia* and *Zea* have shown that a wall is absent at the chalazal end of the synergid. This feature facilitates the movement of the male gamete into the egg and central cell. In most of the plants studied one of the synergids degenerates before fertilization (Jensen & Fisher 1968). The significance of this phenomenon is still an enigma.

Central Cell

The central cell is the largest, binucleate cell of mature embryo sac. The two polar nuclei fuse to form secondary diploid nucleus. One of the sperms fuses with this diploid nucleus to form the triploid primary endosperm nucleus. The central cell is highly vacuolate and is surrounded by a thin layer of cytoplasm rich in many cell organelles. This cell is considered as a site of intense metabolic activity on the basis of the occurrence of both rough and smooth ER. An increase in smooth tubular ER is observed immediately after fertilization in *Epidendrum scutella* and *Petunia*. In *L. usitatissimum* loose whorls of RER associated with vacuoles, homologous to microbodies are present.

Plastids are present in large quantity in the central cell (see Jensen 1965a, Cocucci & Jensen 1969a, Schulz & Jensen 1973, figure 4A, Newcomb 1973, Cass & Karas 1974, Maze & Lin 1975, Schulz & Jensen 1977, Rifot 1978), and are completely filled with starch (van Went 1970a, figure 4B). A few plastids in *Crepis tectorum* have extremely dense stroma (Godineau 1971). Large amyliferous plastids are present in *Linum catharticum* (D' Alascio Deschamps 1973). Chloroplasts have been reported to occur in the cytoplasm of *Capsella*. The plastids of cotton have deposits of phytoferritin and carotene (Jensen 1965b).

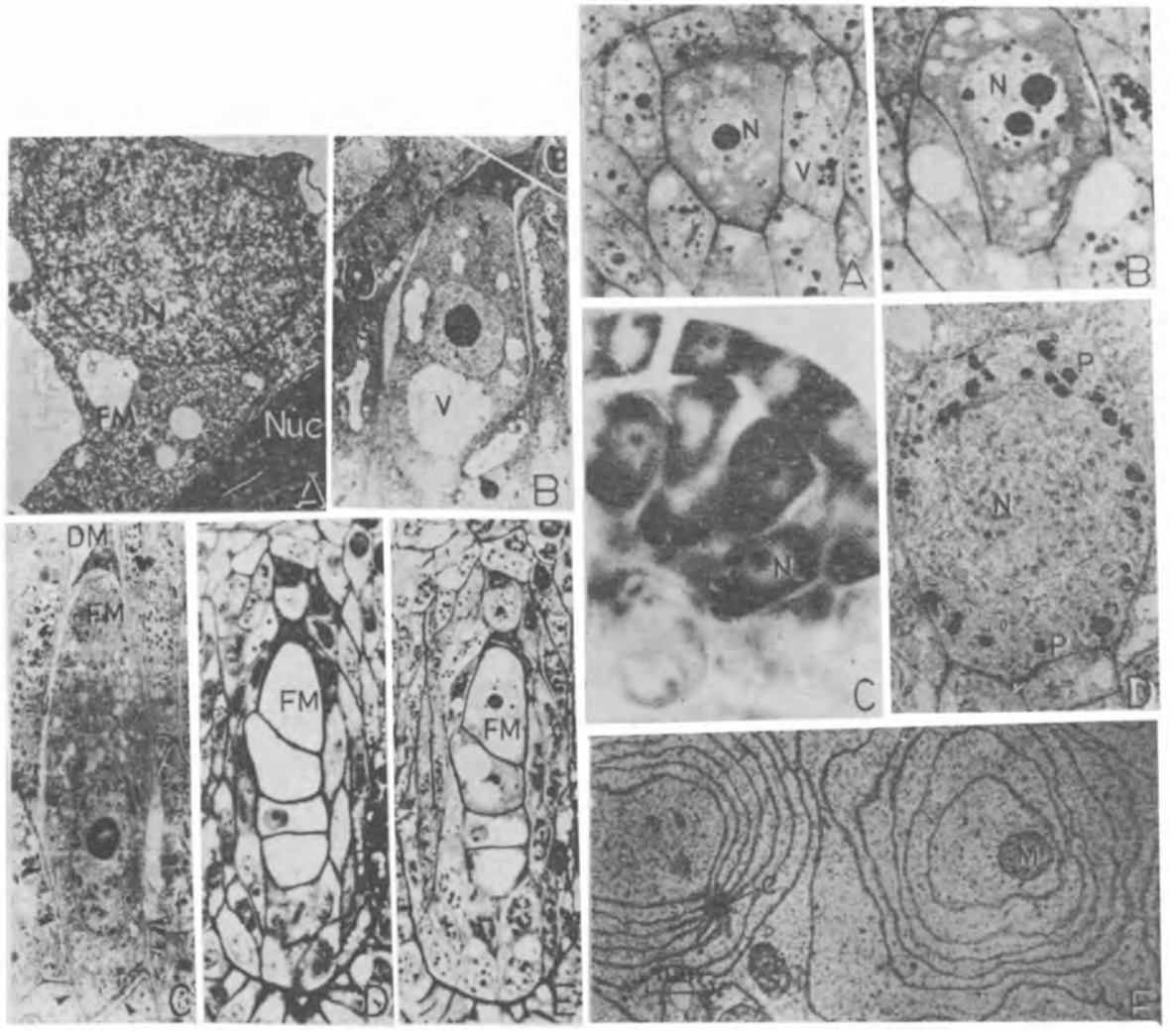
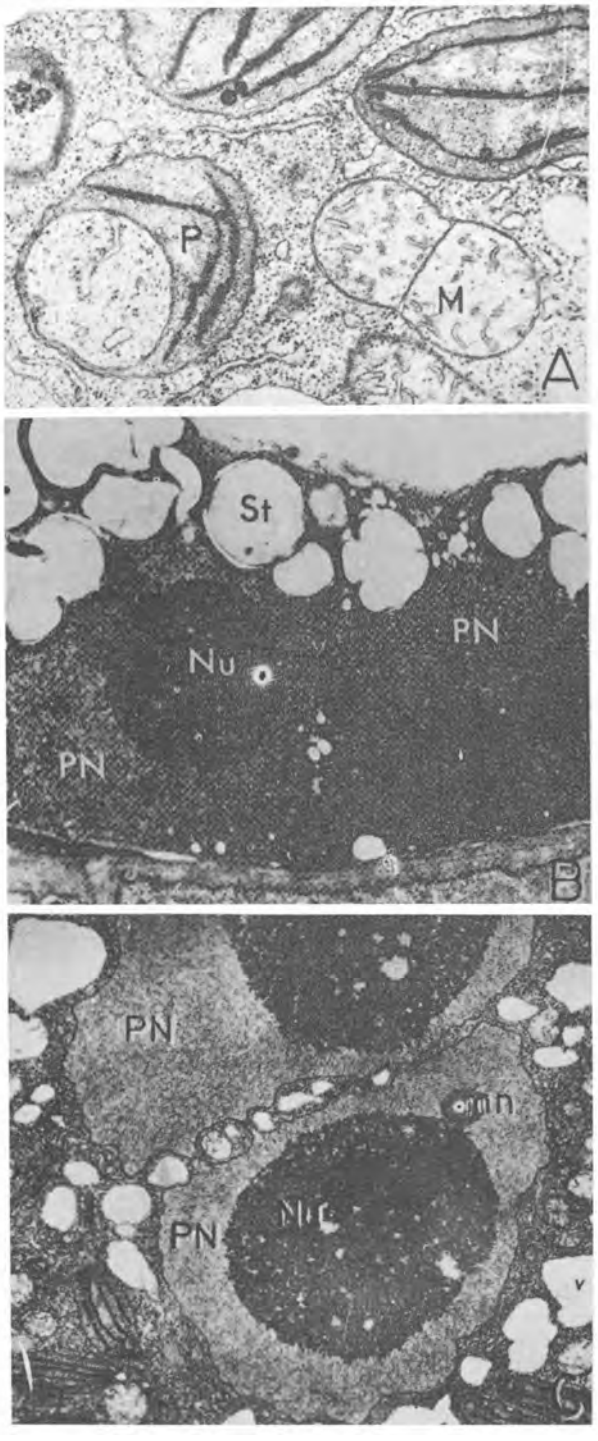


Figure 1A–E Archesporial Cell & Megasporocyte **A, B,** Archesporial cells nearing meiosis in *Ornithogalum caudatum*, stained for nucleic acids and carbohydrate with methylene blue–azure II and basic fuchsin. Very dense cytoplasm, extensive vacuolation (V) & big nucleus (N) are evident (After Tilton 1981b); **C,** Archesporial cells stained for RNA with azure B after DNAase treatment in *Stellaria media*. Large nuclei (N) are present in the archesporial cells. Cytoplasm is deeply basophilic (After Pritchard 1964); **D,** Plastids (P) with starch, after Thiery reaction encircling meiocyte nucleus (N) at a later stage of prophase I in *Epipactis* (After Bednara et al. 1981) **E,** The encapsulation of cytoplasm in *Lilium longiflorum* during prophase in megaspore mother cell. Here the membranes often appear associated with a membrane centre (C) and exclusively multiple membraned inclusions are formed. Mitochondria (M) invested by the membrane (*After Dickinson & Andrews 1977*)

Figure 2A–E Functional Megaspore; **A,** Electron micrograph of an enlarged functional megaspore (FM) and degenerating nucellar cells (Nuc) in *Helianthus annuus*. A big nucleus (N) is present in the functional megaspore (*After Newcomb 1973*); **B,** Embryo sac mother cell with large vacuole (V) in chalazal part in *Stellaria media (after Kudlicka et al. 1981)*; **C,** Finely vacuolate cytoplasm of enlarging functional megaspore (FM) in *Zea mays*. Arrow heads indicate locations of plasmodesmata in chalazal walls. Non-functional megaspore (DM) adjacent to functional megaspore is also evident *(after Russel 1979)*; **D, E,** Serial sections of linear tetrad of haploid megaspores resulting from meiosis in *Ornithogalum caudatum*. Chalazal pair larger than micropylar and of chalazal pair chalazal most is largest. In most instances this one becomes functional megaspore (FM). Vacuolation of functional megaspore is well underway and demonstrates polarized distribution thereof *(after Tilton 1981b)*



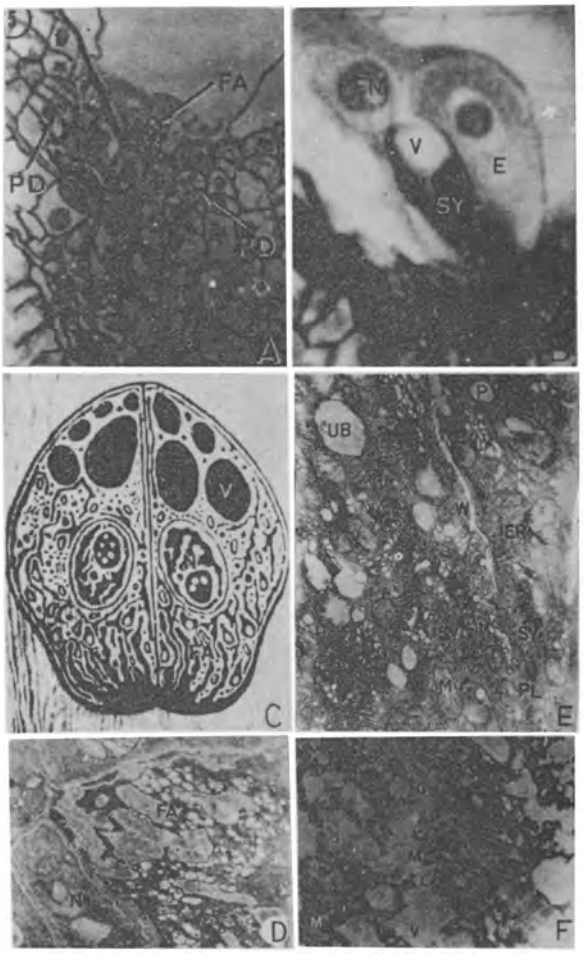


Figure 4A–C Central Cell; **A,** Cup–shaped plastids (P) and a dividing mitochondria (M) in the central cell cytoplasm of *Capsella bursa-pastories*. The cytoplasm in the pocket of the plastid contains short, vesiculate pieces of rough endoplasmic reticulum and is less dense than surrounding cytoplasm *(after Schulz & Jensen 1973);* **B,** Cross-section through the micropylar part of the mature central cell showing the two polar nuclei (PN), nucleolus (Nu) before fusion and plastids of the mature central cell completely filled with starch grains (St) in *Petunia hybrida (after Van Went 1970a);* **C,** The polar nuclei (PN). Each has one large nucleolus (Nu) and a smaller micronucleolus (Mn) in *Capsella bursa-pastoris*. Vacuoles (V) are present around the two polar nuclei *(after Schulz & Jensen 1973)*

Mitochondria are abundant in many taxa. However in *Petunia* and *L. catharticum* their number is low. The central cell mitochondria of *Gossypium* resemble those found in the synergids; contain five or six cisternae and are concentrated in the cytoplasm lining the wall. In *Crepis tectorum* they are found in the perinuclear as well as in the parietal cytoplasm. The dictyosome produce many vesicles. An increase in dictyosome activity is noticed immediately after fertilization in *Petunia* (van Went 1970a). Spherosomes are abundant in cotton. In *Plumbago zeylanica* microtubules are observed in the central cell cytoplasm adjacent to the egg wall.

Polar Nuclei

The two central cell nuclei before they fuse are called the polar nuclei. Each polar nucleus contains a large, dense nucleolus. Micronucleoli have been reported to occur in the polar nuclei of *Capsella bursa-pastoris* (Schulz & Jensen 1973, figure 4C) and barley (Jensen 1973). In cotton each polar nucleolus contains several nucleolar vacuoles. The nuclear membrane is lobed and continuous in many places with RER. In *Spinacia oleracea*, during central cell development, nuclear extensions develop and are connected with each other (Wilms 1981a). In *Capsella bursa-pastoris* peripheral portion of the nucleolus consists of dense granules and fibrillar elements suspended in an amorphous matrix (Schulz & Jensen 1973). The core of the nucleolus is predominantly fibrillar in nature. In *Linum usitatissimum* near the periphery of the central cell nucleus some cytoplasmic enclosures contain plastids, mitochondria and folds of ER.

The time of fusion of the two polar nuclei varies in different taxa. For example the two nuclei fuse completely before fertilization in *Capsella* (Schulz & Jensen 1968b) whereas in *Gossypium* the fusion begins but is not completed until the arrival of the male nucleus. In a few plants the two polar nuclei fuse only after the arrival of the male nucleus. At the start of fusion, the outer nuclear envelopes evaginate, make contact at several points along their facing surface and fuse. This is followed by fusion of inner nuclear membrane leading to the formation of nuclear bridges. Cytoplasm containing organelles even are trapped between these bridges. The nucleoplasm of two nuclei merge completing the nuclear fusion.

A high nucleolar RNA content in the young polar nuclei has been observed in *Vanda* (Alvarez & Sagawa 1965), *Limnophyton obtusifolium* (Shah & Pandey 1977), *Nicotiana rustica* (Sehgal & Gifford 1979), *Paspalum longifolium* (Yu & Chao 1979) and *Argemone mexicana* (Bhandari et al. 1980). The nucleoli of the polar nuclei show intense reaction for RNA (Georgieva 1965) and nuclei for Feulgen reaction (*see* Pritchard 1964, Jensen 1965b, Newcomb 1973). In *Nicotiana tabacum* diffuse chromatin is present in the nuclei of central cell (Bannikova et al. 1981).

Central Cell Wall Ingrowths

In the central cell of many angiosperm plants wall projections are present. In *Euphorbia helioscopia* pectocellulosic wall ingrowths are present towards the antipodal pole and near the egg apparatus (Gori 1977). In *Jasione montana* due to heavy deposition of cutin over most part of embryo sac, wall ingrowths are present in the area of maximum diameter especially near the egg apparatus (Berger & Erdelska 1973). In *Glycine max* at the micropylar end many inward extensions occur between the embryo sac wall and the egg apparatus (Folsom & Peterson 1984). In this area only small plaques of cuticular material are present between the central cell and the surrounding integumentary cells. Similarly in *Zea* (Diboll & Larson 1966), *Crepis* (Godineau 1971), *Stellaria* (Newcomb & Fowke 1973), *Helianthus* (Newcomb & Steeves 1971), and *Linum* (Vazart 1971) wall ingrowths are present around the egg appartus. Similar wall projections are present in the central cell wall adjoining the nucellus in *Gossypium* and *Aquilegia*. In *Capsella* wall ingrowths are present in the cell near the chalazal region in the young megagametophyte but these projections are

Figure 3A–F Egg Cell and Synergids; **A;** Longitudinal section through relatively mature egg of *Plumbago* having conspicuously branched filiform apparatus (FA). Two regions of periclinal cell division (PD) in nucellus are also shown *(after Cass 1972)*; **B,** Egg (E) synergid (SY) and primary endosperm nucleus (PEN) of *Stellaria media,* stained for polysaccharides, proteins and DNA with the PAS reaction, naphthol yellow S, and the feulgen cytoplasmic. The egg and primary endosperm shows high cytoplasmic and nucleolar protein levels. The synergid cell shows PAS positive staining *(after Pritchard 1964)*; **C,** Diagrammatic representation of synergids showing polarity of nucleus (N) and chalazal vacuoles (V) *(courtesy of Prof. W A Jensen, Berkeley California.;* **D,** Filiform apparatus (FA) showing the ingrowths of the cytoplasm in *Aquilegia formosa (after Vijayaraghavan et al. 1972)*; **E,** Micropylar region of two sister synergids (SY) of *Aquilegia formosa.* Prominent wall (W) is present which thins down to plasmalemma (PL). There are many plastids (P), Dictyosomes (D) and variously shaped mitochondria (M) with cristae of different sizes. The endoplasmic reticulum (ER) is closely associated with the wall. There are ribosomes scattered in the cytoplasm and many bounded vesicles of unknown bodies (UB) *(after Vijayaraghavan et al. 1972)* **F,** The common wall (CW) between two contiguous haustoria in the micropyle of *Cortaderia* sp., bears transfer cell type invaginations on each side. Mitochondria (M), Lipid bodies (L) and Vacuoles (V) are evident in the groundplasm of haustoria *(after Philipson 1981)*

absent in the mature megagametophyte and in *Plantago* they are present close to the synergids (Vannereau 1978).

Functions

(i) The presence of extensive ER, numerous mitochondria, dictyosomes, polysomes, and well-developed plastids, microbodies, chloroplasts in the central cell indicate that it is engaged in intense metabolic activity related to nutrition.

(ii) In many plants the central cell due to well developed wall projections acts as conduit for nutrients from the surrounding tissue.

(iii) Plasmodesmata connections between the central cell and egg apparatus are responsible for the nutrition of the egg from the central cell and also gamete transfer to the central cell from the synergids.

(iv) The central cell cytoplasm contains starch and lipid reserves. In *Capsella* these metabolites are quickly utilized during the early development of the endosperm. The presence of spherosomes in the central cell of *Capsella* indicates the initiation of the conversion of stored fats to sugar to supply energy for subsequent growth and development.

(v) The presence of a large nucleolus in the polar nuclei indicates a high rate of protein synthesis. There is a correlation between nucleolar volume and the amount of protein synthesis in the cell. The large nucleolar size indicates also a high rate of ribosome production. The nucleolus is the site of ribosomal RNA synthesis. (see also, Kapil & Bhatnagar 1981).

Antipodal Cells

The antipodal cells are probably the most variable cells in a megagametophyte. In many taxa these cells are ephemeral. In *Jubaeopsis caffra* antipodes degenerate shortly after formation of secondary nucleus and traces of them are left when synergids are fully differentiated (Robertson 1976). Occasionally in a few taxa they increase in size and persist up to the globular proembryo stage (Vijayaraghavan & Usha Bhat 1980).

In many plants persistent antipodal cells show an increase in size, number and in nuclear DNA content. In *Dendromecon rigida* antipodal cells continue to divide during and after fertilization so that there are 30-50 cells after fertilization (Berg 1961). Similarly in *Podolepis jaceoides*, after fertilization 11-15 antipodal cells are present (Davis 1961). The mature embryo sac of barley, after fertilization, contains about 100 antipodal cells (Cass & Jensen 1970). The antipodal cells are 4 and 8 respectively in *Eleusine africana* and *E. compressa*. In *E. africana* each cell is 3-4 nucleate whereas in *E. compressa* it is 1 or 2 nucleate (Chikkannaiah & Mahalingappa 1975). In *Anemone pavonina* nuclei of the antipodals differ in size and number in different stages of the development. Initially they are uninucleate but later they undergo mitotic divisions and multinucleate antipodals result (Ünal 1978). The antipodal cell nuclei also undergo an increase in volume which is associated with DNA synthesis. This increase in DNA concentration is due to endomitosis or polyteny. In antipodal cell nuclei of *Hordeum vulgare* the DNA level ranges between 6 C and 86C during pollination (Bennett & Smith 1976). In Triticale DNA content of the nuclei increases to 256C after fertilization (Kalstipes 1973). In *Scilla bifolia* the endopolyploid level reaches up to 1024 C in the antipodal celll nuclei which frequently display polytene chromosome (Nagl 1976). The antipodal cell in *Scilla decidua* show endopolyploid level up to 512 C in their nuclei (Frisch & Nagl 1979). In various species of *Ranunculus* antipodal nuclei attain different levels of endopolyploidy after fertilization (Bohdanowicz & Turala-Szybowskia 1985, 1987, Turala-szybowskia & Wedzony 1981, Vijayaraghavan & Bhat 1980).

In *Zea mays* during further divisions in antipodal cells, the walls of the newly formed cells are incomplete thus leaving protoplasmic continuities between these cells and formation of multinucleate protoplasts (Diboll & Larson 1966). Ramaswamy and Govindappa (1981) reported a peculiar behaviour of the antipodal cells in Ericaulaceae. The separating walls of antipodals completely disappear and an antipodal-cyst is formed due to the coalescence of the three naked protoplasts and subsequent fusion of nuclei. In *Linum usitatissimum* the antipodal complex is formed with four antipodal cells separated by incomplete walls (Vazart 1969). Later these discontinuities also disappear and result in the formation of a syncytium.

The wall which surrounds the antipodal cell is thick and continuous in *Linum catharticum* (D'Alascio-Deschamps 1973). In *Aquilegia vulgaris* each cell has a relatively thin wall surface except at the base. The partition wall that separates the two antipodal cells and antipodal and central cell appears to be composed of elongate fibrils arranged in the longitudinal direction (Rifot 1973). In *Gasteria verrucosa* the cell wall between the antipodes is thin (Willemse & Kapil 1981) and in *Jasione montana* is surrounded by distinct electronlucent walls reminiscent of callose (Berger & Erdelska 1973).

In *Crepis tectorum* the antipodal cells are connected with each other and with the central cell by numerous plasmodesmata. A few plasmodesmata are also present in the antipodal wall at the chalazal region (Godineau 1971). Numerous plasmodesmata are present in the interantipodal regions in *Aquilegia vulgaris* (Rifot 1971, 1973) but no plasmodesmata are present in the wall of the chalazal region. In *Jasione montana* (Berger & Erdelska 1973), and *Gasteria verrucosa* (Willemse & Kapil 1981a) numerous plasmodesmata

are reported to occur between the interantipodal walls and also in the wall facing the central cell. In *Spinacea oleracea* plasmodesmata are present in the chalazal region of antipodal cells (Wilms 1981a).

Wall Ingrowths

In *Linum usitatissimum,* wall ingrowths are present at the basal region of the antipodal complex. These ingrowths increase the surface area of absorption and help the embryo sac to absorb materials produced by decomposition of the nucellus (Vazart 1969). In *Aquilegia vulgaris* antipodal wall outgrowths are cellulosic and thick in the chalazal region (Rifot 1971, 1973). Numerous mitochondria are present near the wall outgrowths. In *Conium maculatum* (Dumas 1978) and *Paspalum longifolium* (Yu & Chao 1979) the walls in contact with the nucellus have well developed finger-like projections. In *Nigella damescena* the antipodal cell wall ingrowths are long, branched and spread fan-like into the antipodal cytoplasm indicating that they act as conduit of metabolites. The interantipodal wall ingrowths are small, papillate and occur on both sides of the antipodes which imply exchange of metabolites between them. (Vijayaraghavan et al. 1988).

The cytoplasm of antipodal cells has abundant mitochondria, dictyosomes, plastids and well developed endoplasmic reticulum in several taxa — *Zea mays* (Diboll & Larson 1966), *Epidendrum scutella* (Cocucci & Jensen 1969a), *Crepis tectorum* (Godineau 1971), *Aquilegia vulgaris* (Rifot 1971), *Linum catharticum* (D'Alascio-Deschamps 1973), *Helianthus annuus* (Newcomb 1973), *Stipa elmeri* (Maze & Lin 1975) and *Spinacia oleracea* (Wilms 1981a). No dictyosomes have been observed in the cytoplasm of mature antipodal cells in *Gastria verrucosa* (Willemse & Kapil 1981a). In *Nicotiana rustica* these cells, are devoid of ER and dictyosomes; have abundance of mitochondria and plastids (Sehgal 1980). Endoplasmic reticulum and dictyosomes are scarce or absent in the cytoplasm of antipodal cells in *Capsella bursa-pastoris* (Schulz & Jensen 1971). In *Crepis tectorum* (Godineau 1971) endoplasmic reticulum runs parallel to the cell wall of antipodal cells whereas in *Aquilegia vulgaris* a few strands of endoplasmic reticulum are localized in the periphery of the antipodal wall and a few ER remain perinuclear (Rifot 1971).

The cytoplasm is rich in ribosomes as in *Linum usitatissimum* (Vazart 1969), *L. catharticum* (D'Alascio deschamps 1973), *Stipa elmeri* (Maze & Lin 1975), *Gasteria verrucosa* Willemse & Kapil 1981a) and *Spinacia oleracea* (Wilms 1981a). In *Epidendrum scutella* (Cocucci & Jensen 1969a) and in *Capsella bursa-pastoris* (Schulz & Jensen 1971), the antipodal cells contain a large nucleus with a single nucleolus and uniformly distributed chromatin material whereas in *Linum usitatissimum* (Vazart 1969) and *L. catharticum* (D'Alascio Deschamps 1978) antipodal cell nuclei have large compact nucleolus with nucleoplasm filled with abundant fibrillar material.

In *Epidendrum scutella* polygonal, crystal-like, bodies are present inside the plastids of antipodal cells (Cocucci & Jensen 1968a). These bodies consist of phytoferritin and seem to be analogous to the paracrystalline proteinaceous deposits found in the enlarged RER of the integumentary cells of *Diplotaxis erucoides* (Cresti et al. 1974). They are perhaps involved in nutrition.

A PAS-positive wall is reported in the antipodal cells of *Hordeum vulgaris* (Cass & Jensen 1970), *Zephyranthes rosea* and *Lagenaria vulgaris* (Malik & Vermani 1975), *Argemone mexicana* (Bhandari et al. 1980) *Papaver nudicaule* (Randall-Olson & Cass 1981) and *Papver somniferum* (Bhandari & Bhargava 1983). This reaction confirms the polysaccharide nature of cell wall of the antipodes.

The antipodes of *Vanda* show a very low concentration of insoluble polysaccharides, proteins and RNA (Alvarez & Sagawa 1965). In *Dipcadi montanum* (Syamasundar & Panchaksharappa 1976), *Farsetia hamiltonii* and *Eruca sativa* (Prasad 1977), *Paspalum longifolium* (Yu & Chao 1979), *Argemone mexicana* (Bhandari et al., 1980) *Linaria bipartita* (Kallarackal & Bhatnagar 1980) and *Ranunculus sceleratus* (Vijayaraghavan & Bhat 1980), the antipodal cells are rich in total proteins. Cytoplasm of antipodal cells is PAS- positive in *Dipcadi montantum* but is PAS-negative in *Linaria bipartita* and in *Ranunculus sceleratus* (Vijayaraghavan & Bhat 1980), the antipodal cells are rich in total proteins. Cytoplasm of antipodal cells is PAS-positive in *Dipcadi montantum* but is PAS-negative in *Linaria bipartita* and in *Ranunculus sceleratus.* The cytoplasm of antipodals is rich in histones in *Stellaria media* (Pritchard 1964) and *Ranunculus sceleratus* (Vijayaraghavan & Usha Bhat 1980). The persistent antipodal cells possess a high rate of metabolism as evidenced by the enormous increase in proteins, histones, DNA and RNA, after fertilization in *Ranunculus sceleratus* (Vijayaraghavan & Bhat, 1980). The antipodal cell cytoplasm in *Stipa elmeri* contains lipid bodies (Maze 1975). In the ovules of *Cypripedium* starch grains appear before fertilization and accumulate mainly in the chalazal end of the embryo sac (Zinger & Poddubnay Arnoldi 1966). Little starch is present in barley antipodal cells (Cass & Jensen 1970).

In *Dipcadi montanum* the antipodal cells are persistent, and contain a giant nucleus that is densely Azure-A positive due to increase in DNA content (Syamasundar & Panchaksharappa 1976). In *Paspalum longifolium* endomitosis results in high DNA content and shows intense staining for Azure B (Yu & Chao

1979). In *Linaria bipartita* the nuclei of the antipodal cells show maximum staining for DNA due to endomitosis and polyteny. In *Ranunculus sceratus* also, Feulgen staining is more intense in the nuclei of antipodals than those of synergids (Vijayaraghavan & Usha Bhat 1980).

Localization of enzymes in three orchids *Cypripedium, Dendrobium* and *Calanthe,* revealed that the antipodal cells are rich in peroxidases, oxidases, sulfhydryl compounds (Zinger & Poddubnaya Arnoldi 1966). Maximum activity of ascorbic acid was noted in *Cypripedium.* In Coix lacryma-jobi the antipodal cells exhibit a gradient of ascorbic acid concentration (Bhat & Shah 1973). Antipodal cells have maximum activity of enzymes like peroxidases, succinate dehydrogenases, cytochrome oxidases and ATPase in *Zephyranthes rosea* (Malik & Vermani 1975). The high rate of respiratory activity due to respiratory enzymes may be involved in regulating the growth and differentiation of embryo sac.

Functions

(i) The presence of plastids, dictyosomes, and motochondria, in antipodal cells, indicate that they are engaged in intense metabolic activity. Antipodal cells may be sites of active protein synthesis as evidenced by extensive endoplasmic reticulum and high density of ribosomes. The presence of large amounts of enzymes like ascorbic acid, peroxidases, dehydrogenases, oxidases and of RNA, DNA and proteins indicates high physiological activity which is responsible for growth, differentiation and nutrition of the embryo sac.

(ii) The presence of prominent wall projections in the chalazal region suggest that antipodal cells absorb large amount of nutrient substances from the surrounding tissue.

(iii) Numerous mitochondria are present near the outgrowths of the wall and would provide energy for the active transport of nutrient substances. The plasmodesmata connections between the antipodal cells and between the antipodal cells and central cell would aid in transportation of metabolites from the antipodal cells to the central cell.

(iv) Antipodal cells act as sites of storage of starch and lipids needed for developing endosperm and embryo.

(v) The active metabolic synthesis is associated with high level of polyploidy. These cells may also have a role in the transfer of nutrients during early embryogenesis.

Acknowledgements

We wish to express our sincere gratitude to Professor H Y Mohan Ram for critically going through the manuscript and offering many valuable suggestions for improvement of the text. Thanks are due to Drs W A Jensen, J L van Went, W Newcomb, Patricia Schulz, H G Dickinson, D D Cass, J Bednara, K Kudlicka, M N Pritchard, S D Russel, and V R Tilton for providing illustrations from their publications. We sincerely acknowledge the help of Dr Tripat Kapoor, Dr Nidhi Gautam and Miss Gayatri Misra for constant encouragement. We thank Mr R K Gupta for meticulous typing of the manuscript.

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