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The Egg Cell: Development and Role in Fertilization and Early Embryogenesis

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INTRODUCTION

Flowering plant reproduction is unusual in many features, as evidenced by the different topics discussed in this issue of THE PLANT CELL. The egg cell of flowering plants in particular displays several unique features. First, the egg cell is an integral part of the several thousands of cells forming the ovule, and it cannot be released without the aid of enzymes or microdissection. The egg cell appears to be part of a functional assemblage of cells that are fragile in isolation. Surrounding the egg cell in situ are the two synergids (collectively forming the egg apparatus) and the adjacent central cell, as shown in Figure 1. These are almost always derived from the same initial meiotic cell as the egg cell. The surrounding cells appear to provide nutrition for the egg cell and are involved in the positioning of the sperm cells to the precise site where gametic fusion occurs.

Different developmental programs are invoked in both the egg and central cells by the fusion of each with a sperm cell in an event characteristic of flowering plants known as "double fertilization." During this event, one sperm nucleus fuses with the egg nucleus to form the zygote, whereas the other sperm nucleus fuses with the two (or more) central cell nuclei, resulting in the formation of the nutritive endosperm.

The developmental potential, biochemical identity, and characteristics of the egg that adapt it to serve as a cell that readily accepts the genome of the sperm are poorly understood. Establishing an in vitro reproductive system comparable to those in animal biology is an attractive but apparently complex goal to achieve. Although the isolation of living eggs in flowering plants is not now considered unusual, the first published account did not emerge until the late 1980's. Only recently has a culture medium been described in which eggs and sperm cells will fuse in vitro without the addition of polyethylene glycol or the use of electroporation (J.-E. Faure, C. Digonnet-Kerhoas, and C. Dumas, unpublished results; Dumas and Mogensen, 1993, this issue).

The current knowledge of egg formation and differentiation and the in vivo behavior of the embryo sac will be the emphasis of this review. More complete discussions of the organization and function of embryo sac cells are available elsewhere (Jensen, 1974; Kapil and Bhatnagar, 1975, 1981; van Went and Willemse, 1984; Willemse and van Went, 1984; Huang and Russell, 1992a; Russell, 1992; Reiser and Fischer, 1993, this issue).

FORMATION AND DEVELOPMENT OF THE EGG CELL

The egg originates from a single diploid cell known as the megasporocyte, which is located near the center of the ovule (see Gasser and Robinson-Beers, 1993, this issue; Reiser and Fischer, 1993, this issue). This cell divides meiotically and establishes the lineage that forms the egg cell. Numerous variations of megaspore formation (megasporogenesis) and embryo sac development (megagametogenesis) have been described in flowering plants, as Table 1 shows. The characteristics that vary include the number of mitotic divisions, the placement of the resulting nuclei, the patterns of cellularization, and the organization of the mature embryo sac. Innumerable minor variations also occur. These processes are still being examined from morphological, developmental, genetic, and evolutionary perspectives (Battaglia, 1989).

The mature embryo sacs of different species frequently have dissimilar levels of genetic heterogeneity, depending on whether one, two, or all four megaspore nuclei contribute to their formation. The functional organization of the mature embryo sac—that is, of the cells of the micropylar end of the embryo sac (Figure 1)—is remarkably similar among species that vary in their patterns of megasporogenesis and megagametogenesis. The only major variants are the embryo sacs that develop according to the *Peperomia* type, which forms only one synergid, and those that conform to the *Plumbago* and *Plumbagella* types, which do not form any synergids.

The formation of the egg cell and one of the polar nuclei can be traced from the four-nucleate stage of embryo sac development, when two nuclei migrate to the micropylar end of the embyro sac and two to the chalazal end. One of the two micropylar nuclei becomes located closer to that end than the other, as has been observed in a number of angiosperms (reviewed in Folsom and Cass, 1990). The nucleus closest to the micropyle is believed to give rise to the two synergid nuclei, whereas the other micropylar nucleus divides to form the two reproductive nuclei—the egg nucleus and one of the polar nuclei of the central cell.

Some species appear not to possess such distinct differences between micropylar cells at their inception. In *Ranunculus*, the nuclei are located equally close to the micropylar end of the embryo sac (Bhandari and Chitralekha, 1989), and in *Lilium longiflorum*, the egg cell is reported to be difficult if not impossible to distinguish from the synergid cells before fertilization (Janson, 1992); thus, there is some question

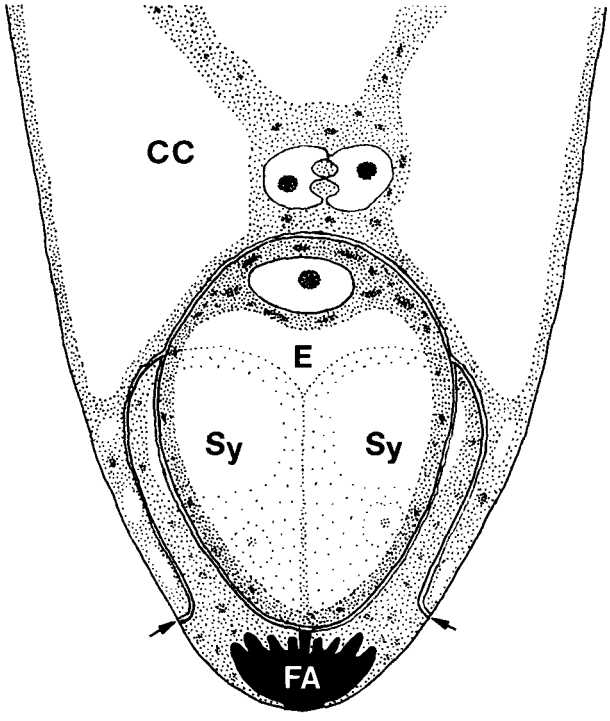

Figure 1. The Female Germ Unit.

The female germ unit—the assemblage of cells active in sexual reproduction in flowering plants—consists of the egg cell (E), central cell (CC), and the two synergids (Sy). A filiform apparatus (FA) composed of cell wall ingrowths marks the micropylar end of the two synergids and is the site of pollen tube entry. The egg cell will unite with a sperm cell to form the zygote and embryo, whereas the polar nuclei that are in contact with each other in the central cell will unite with the other sperm nucleus to form the primary endosperm nucleus and endosperm. Small arrows indicate points of attachment of the synergid to the embryo sac wall forming so-called synergid "hooks" or apical pockets of central cell cytoplasm.

as to whether there is a predetermined egg cell in the latter species. In most species, including *Ranunculus* and *Lilium*, the two spindles at the micropylar end of the embryo sac become organized differently: one spindle is oriented transversely and will presumably form the two synergid nuclei, and the other is oriented longitudinally to obliquely and will presumably form the egg nucleus and one of the polar nuclei of the central cell (Folsom and Cass, 1990).

Although there is apparently no genetic predisposition for any of the embryo sac nuclei to form the egg cell, the nucleus positioned slightly chalazally to the two most micropylar nuclei always assumes this role. At what point the cells of the embryo sac become committed to the reproductive pathway is unclear; however, the staggered placement of the two micropylar nuclei at the four-nucleate stage suggests an early distinction between these two nuclei that may become fixed as the final cycle of mitosis occurs and the embryo sac undergoes cellularization.

CELLULARIZATION

Cellularization immediately follows the final mitotic division of embryo sac formation. A total of eight nuclei are usually present, although "strike" (nondivision of nuclei) may reduce this number in the chalazal end of the embryo sac; that is, the cells at the antipodal end of the embryo sac may never be formed. The four nuclei at the micropylar end of the embryo sac are those that form the reproductively functional cells of the embryo sac. It is not yet known how these nuclei cellularize. As Figure 2 shows, cellularization may involve two, three, or four cell plates, forming three different models of embryo sac development.

Two consistent features in all of the models of embryo sac cellularization include the formation of one cell plate between the two synergids and the formation of a second cell plate delimiting the central cell from the cells of the egg apparatus (Figure 2). The phragmoplasts that form the synergid common wall and the chalazal egg apparatus wall, respectively, appear to originate from interzonal fibers remaining from the final mitotic division. The formation of cell plates between nonsister nuclei is, however, currently unresolved.

According to the four-cell plate model, phragmoplasts are organized between the egg nucleus and each of the two adjacent synergid nuclei to form the remaining two cell plates (Figure 2A). Bhandari and Chitralekha (1989) propose, however, that a single, curved cell plate partitions the egg nucleus from the two synergid nuclei in *Ranunculus*; in this case, cellularization would involve just three cell plates (Figure 2B; also, see Folsom and Cass, 1990). Cass et al. (1985, 1986) propose that in barley, only two cell plates form the micropylar half of the embryo sac: the chalazal egg apparatus wall bifurcates to enclose the egg cytoplasm from the adjacent synergid nuclei (Figure 2C).

Although the phragmoplast that forms between sister nuclei appears to originate directly from interzonal microtubules of the previous spindle, the phragmoplast of nonsister nuclei may arise de novo. One mechanism by which this may occur is suggested by the work of Brown and Lemmon (1991) on the division of microsporocytes in orchids. Cell walls formed during microsporogenesis, as in other gametophytic cells, lack preprophase bands (PPBs); instead, the site of cell partitioning appears to be determined by "cytoplasmic domains" consisting of microtubules emanating from individual nuclei. Each field of microtubules claims a region of cytoplasm in which the microtubules extend in one pattern of polarity. The phragmoplast forms where microtubules of different cytoplasmic domains and opposite polarities interact, apparently determining the plane of cytokinesis and establishing the location of the cell plate (Brown and Lemmon, 1991).

Table 1. Variability in Normal Megasporogenesis in Angiosperms

Traditional Nomenclature	Revised Nomenclature[a]	Examples
Conventional Patterns of Megasporogenesis		
Monosporic	Monokaryosporic	Polygonum, Oenothera, and Schisandra types
Bisporic	Dikaryosporic	Melica (= Allium) and Hyacinthoides (= Endymion) types
Tetrasporic	Tetrakaryosporic	Adoxa, Euphorbia dulcis (= Fritillaria), Peperomia, Penaea, Plumbago, and Plumbagella types
Unconventional Patterns Based on Breakdown of Cell Wall Partitions		
Monosporic, functionally bisporic	Dikaryosynsporic	*Convallaria*
Monosporic, functionally tetrasporic	Tetrakaryosynsporic	*Maianthemum* (early degeneration of abortive megaspore nuclei), *Leontodon* (late degeneration)
Unconventional Patterns Based on Degeneration of Megaspore Nuclei		
Bisporic, functionally monosporic	Ex-di-monokaryosporic	Podostemaceae
Tetrasporic, functionally monosporic	Ex-tetra-monokaryosporic	*Clintonia*
Tetrasporic, functionally bisporic	Ex-tetra-dikaryosporic	Limnanthaceae
Tetrasporic, functionally trisporic	Ex-tetra-trikaryosporic	Occasional species

[a] After Battaglia (1983). This terminology emphasizes the nuclear nature of early embryo sac formation.

Because cellularization in embryo sacs also occurs in the absence of PPBs, cytoplasmic domains in the egg and synergid cytoplasm may possibly participate in forming the egg cell wall. Microtubules emanating from the egg nucleus would presumably establish the cytoplasmic domain of the egg cell and interact with those microtubular fields emanating from other nuclei, particularly those of the synergids. This interaction of microtubules from both the synergids and the egg cytoplasm could conceivably form a single, curved phragmoplast like that observed around the egg nucleus or could direct the growth of a bifurcated cell plate.

After the completion of cell wall formation, the egg and synergids are poorly differentiated from each other. Each cell is small and wedge shaped, with a flattened chalazal wall (Cass

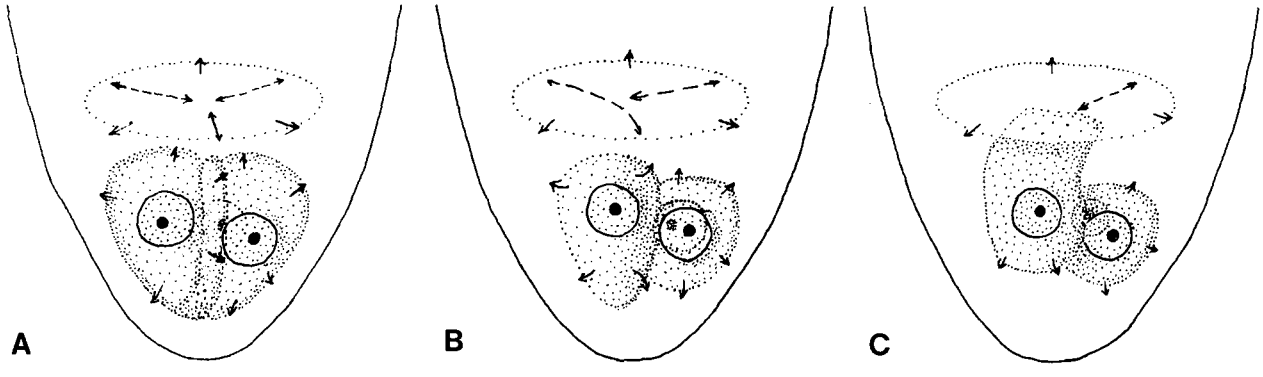

Figure 2. Three Models of Cellularization in the Angiosperm Female Germ Unit.

Common to all three models are: (1) a vertical phragmoplast between the two synergid nuclei (right and in the back) forming the synergid common wall and (2) a horizontal phragmoplast between the egg nucleus (left) and one polar nucleus (not shown) forming the chalazal egg apparatus wall. The formation of the egg cell wall differs in all three models. Small arrows indicate the direction of phragmoplast expansion, and dashed lines indicate planes of intersection with the horizontal cell plate.

(A) Four phragmoplasts form between the micropylar nuclei in the expected pattern of cytokinesis involving four nuclei. This pattern has been reported previously in the light microscopic literature (see Battaglia, 1991) but has not been confirmed with modern methods.

(B) Three phragmoplasts form between the micropylar nuclei in *Ranunculus* (Bhandari and Chitralekha, 1989). A single curved cell plate forms around the egg nucleus, separating it from both synergids simultaneously.

(C) Two phragmoplasts form in barley (Cass et al., 1985, 1986). The cell plate between the egg nucleus and the polar nucleus bifurcates, enclosing the egg cell on all sides.

et al., 1986; Folsom and Cass, 1990) or a slightly indented wall where the central vacuole encroaches into the micropylar cytoplasm (Bhandari and Chitralekha, 1989). At this stage, the egg and synergid cells are similar in size and shape. Each of these cells then expands toward the chalaza, inflating into rounded cells of similar size but different placement (see Figure 1). The synergids are located at the micropylar end of the embryo sac, whereas the egg cell is displaced chalazally by several micrometers and is attached laterally to the embryo sac wall. The surface of the egg cell is shared with the central cell, with part of the micropylar embryo sac wall, and with each of the two synergids (Russell and Mao, 1990). The egg cell and both synergids inflate until the chalazal boundaries of these cells become stretched and their cell walls become discontinuous and "beadlike," consisting of islets of cell wall material separated by segments of closely appressed plasma membrane (Cass et al., 1986).

The egg cell remains morphologically similar to the synergids until cell wall ingrowths form at the micropylar pole of the two synergids, forming the distinctive filiform apparatus ingrowths that identify these cells as synergids (Cass et al., 1986). The micropylar walls are essentially unchanged by the chalazal expansion of the egg cell, whereas the attachment sites of the synergids with the embryo sac wall are frequently stretched until they become perpendicular to the exterior of the embryo sac (arrows in Figure 1). The so-called synergid and egg "hooks" (Cass et al., 1986) or "apical pockets" represent areas of sequestered cytoplasm found in embayments of the central cell at the attachment sites of the egg cell and synergids. Dramatic increases in cell size are typical during maturation.

Cellularization closes the coenocytic phase of embryo sac development, but it is perhaps the most poorly understood part of megagametogenesis. A reexamination will ultimately be needed to determine conclusively how cell plates are formed and how cells differentiate to assume their mature function. Approaches such as tubulin localization and three-dimensional computer-assisted reconstruction may be necessary to visualize how phragmoplasts are formed and how cellularization contributes to the differentiation of the egg cell. A remaining problem is that cellularization is extremely rapid in flowering plants; a critical analysis of these models using precise developmental staging will be crucial to determining the validity of these models (see also Battaglia, 1991; Huang and Russell, 1992a).

FEMALE GERM UNIT

As originally proposed by Dumas et al. (1984), the "female germ unit" (FGU) is the minimum complement of cells required to effect double fertilization in vivo. The FGU of a typical angiosperm is composed of the egg cell, two synergids, and the central cell. The components of the FGU attract and receive the pollen tube, cause the sperm cells to be discharged into the receptive part of the female gametophyte, and transport and promote the fusion of one sperm cell with the egg cell and the other sperm cell with the central cell (see Dumas and Mogensen, 1993, this issue).

Prior electron microscopic research has brought acceptance of the concept that the synergids work in cooperation with the egg in the "egg apparatus" (see reviews by Jensen, 1974; Kapil and Bhatnagar, 1975; van Went and Willemse, 1984). The FGU extends the concept of the egg apparatus to include the central cell, which performs the essential function of forming the nutritive endosperm (see Lopes and Larkins, 1993, this issue).

The pattern of cellular contacts between the egg, synergids, and central cell adds further credence to the concept of the FGU. The normal, intact FGU includes (1) narrow micropylar entries that impede the entry of supernumerary pollen tubes that might otherwise cause polyspermy or heterofertilization (Mogensen, 1978); (2) the presence of cytoskeletal elements established within and between cells, which may aid in sperm cell guidance and the migration of the male nuclei; (3) the presence of poorly developed cell walls near the sites of male gametic transfer in the egg and central cells, which promotes cell fusion; and (4) the proximity of the egg and polar nuclei at the time of fusion, which promotes nuclear migration.

The sperm cells are associated with the vegetative nucleus in a complementary "male germ unit" (MGU), which constitutes the functional male reproductive unit of DNA transmission (Russell, 1991; see also Dumas and Mogensen, 1993, this issue). After fertilization, both the male and female germ units undergo significant changes that dissolve their associations and convert the embryo sac environment into one suitable for the development of the next sporophyte generation. The chalazal migration of the primary endosperm nucleus, cytoskeletal changes in the early postfertilization zygote, and the regeneration of cell walls around the zygote are among the most conspicuous indications that the FGU has completed its sexual function.

MATURE, RECEPTIVE EGG CELL

The mature, receptive egg cell is usually similar in shape to the synergids but is typically placed chalazal to them. The synergids occupy the most micropylar location, positioning them advantageously to intercept the pollen tube (Figure 1). The egg cell itself is not penetrated by the arriving pollen tube; instead, the pollen tube pushes into one of the two synergids, ruptures, and then releases the two sperm cells into the synergid, from where they are transferred into the egg and central cell. The cell wall of the egg cell is incomplete over the chalazal one-half to two-thirds of the cell, exposing large areas of egg cell plasma membrane adjacent to the synergids and central cell (Huang and Russell, 1992a).

In most species, the egg and synergid cells are cytologically distinct and strongly polarized. The egg nucleus may be

located chalazally, with a single central vacuole, or, alternatively, it may be located centrally and surrounded by a large number of smaller vacuoles. The egg nucleus remains large and appears regular in form and structure, with a large nucleolus, whereas synergid nuclei are normally smaller, denser, and more irregular in form and may lack or contain small nucleoli.

The large nucleoli of the egg cell may indicate amplification of rDNA and an exceptionally high potential for the production of ribosomes, as in animal oocytes (Alberts et al., 1989). A high density of cytoplasmic ribosomes has been reported in all flowering plant eggs examined to date (for review, see Kapil and Bhatnagar, 1981), most of which are characterized by large nuclei and nucleoli. Polyribosomes are rarely observed in unfertilized egg cells, suggesting that few proteins are actively synthesized in the egg at maturity.

The ultrastructure of the egg cytoplasm varies considerably among the angiosperms. Although most egg cells are poor in Golgi bodies, variability exists in the distribution and abundance of plastids, mitochondria, endoplasmic reticulum, and lipid bodies. Plastids in the egg cell are usually distributed around the nucleus, display variability in size and shape, contain rudimentary lamellae, and sometimes contain starch grains, possibly as a dynamic reserve. The number of plastids reported in the egg varies from 730 in *Plumbago* (Russell, 1987) to 8 to 12 in *Daucus* species that display paternal cytoplasmic inheritance (Boblenz et al., 1990). Mitochondria are abundant, typically spherical to roundly ellipsoidal, and generally perinuclear in distribution, with poorly developed cristae. Estimates of the number of egg cell mitochondria range from 1000 to 2500 in *Impatiens* to 40,000 or more in *Plumbago* (Huang and Russell, 1992a). The abundance of endoplasmic reticulum varies, as does that of lipid bodies. *Plumbago* is the only flowering plant known to contain microbodies in the egg cell (Cass and Karas, 1974), which in other organs would be expected to function as peroxisomes or glyoxysomes.

The micropylar cell wall of the egg contains fibers suggestive of cellulose microfibrils and acidic polysaccharides (Sumner and van Caeseele, 1989). The chalazal cell wall is typically interrupted by areas of plasma membrane contacts and often contains a homogeneous gel-like substance that lacks interlinked fibrillar components. Characteristic electron-dense bodies may also be present at intervals between the FGU cells, occurring specifically at the chalazal end of the degenerate synergid and between the egg and central cell (Russell, 1992; Sumner, 1992).

The microtubular cytoskeleton of the egg cell is densest near the nucleus. In this location, the orientation of microtubules is essentially random. Egg cells labeled for F-actin with rhodamine–phalloidin stain display a random organization of actin bundles that do not appear as common as bundles of microtubules.

Based on ultrastructural characteristics, the normal, unfertilized egg cell appears to contain the potential for a highly synthetic cellular physiology, but it is quiescent in appearance compared to the synergid (Jensen, 1965). This impression of quiescence is confirmed by video observations of living embryo sac cells, in which the egg cell displays the least cytoplasmic activity of the FGU (Huang et al., 1992). Golgi bodies are apparently inactive in vesicle production, suggesting that the Golgi are not actively involved in secretion in the mature, unfertilized egg.

Although the occurrence of synergid-lacking embryo sacs in angiosperms is rare, embryo sacs such as those of *Plumbago* and *Plumbagella* still appear to display the quintessential characteristics of flowering plant embryo sacs, except that the egg and synergid cell functions are apparently combined. The egg cell of these plants represents the only example of the occurrence of a filiform apparatus in any embryo sac cell other than a synergid (Cass and Karas, 1974). The filiform apparatus is organized similarly to those in the synergids of other flowering plants. The egg cell of *Plumbago* is also strongly zonated in the organization of its cytoplasm and, particularly, of its cytoskeleton (Huang et al., 1990, 1993). The micropylar end contains longitudinally aligned microtubules typical of synergids, which terminate adjacent to the filiform apparatus. The middle contains fewer microtubules, and the chalazal end contains numerous randomly oriented microtubules. In this plant, actin is distributed in axially aligned anastomosing bundles that apparently allow the egg to expand in girth but not in length (Huang et al., 1993).

Interestingly, electron-dense bodies similar to those reported in other flowering plants also occur in the egg–central cell boundary of *Plumbago*. These structures are reportedly associated with microtubules and seem to maintain a constant distance between the cells, apparently stabilizing the egg–central cell boundary before fertilization (Cass and Karas, 1974). These remain evident in the region through which gametes enter and appear to persist at least briefly after fertilization (Russell, 1983). Observations using intermediate voltage electron microscopy indicate that the cytoplasm is both thicker and denser in the egg cell than in the central cell (Russell et al., 1989). The egg cell of *Plumbago*, in contrast to the majority of angiosperm egg cells, contains numerous mitochondria, plastids, rough endoplasmic reticulum lamellae, and Golgi bodies and seems, therefore, to be very active physiologically.

RECEIPT OF THE POLLEN TUBE AND TRANSMISSION OF SPERMS

Although abundant information is available on the role of the synergids during fertilization, less is available on the egg cell, which appears to be a more passive participant (Huang and Russell, 1992a; Russell, 1992). In all species studied to date, the pollen tube pushes into one of the two synergids. This, the receptive synergid, degenerates either before pollen tube arrival or concomitantly with tube arrival. In some species, the synergid degenerates even before pollination, but in others the synergid appears to remain intact until at least the time

of pollination or the arrival of the pollen tube. The receptive synergid is readily recognizable after it degenerates, because it lacks a plasma membrane and a tonoplast.

The synergid is unusual in a number of important characteristics: (1) it has characteristic cell wall ingrowths at its micropylar end termed a filiform apparatus (Huang and Russell, 1992a); (2) its ultrastructural appearance reflects an active metabolism with numerous, well-formed mitochondria (Jensen, 1974); and (3) the vacuole contains massive quantities of calcium (Jensen, 1965; Chaubal and Reger, 1990, 1992b). Up to 50% of the dry weight of the synergid vacuole is calcium, according to standardless semiquantitative energy-dispersive x-ray analysis (Chaubal and Reger, 1992a). The synergid is also believed to serve as the source of a chemical signal that directs the pollen tube to enter the micropyle, penetrate one synergid, and release its gametes (Jensen et al., 1985).

Immunogold electron microscopic studies indicate that the electron-dense material between the egg and the central cell contains high levels of actin (B.-Q. Huang and S.D. Russell, unpublished data). In normal embryo sacs, twin actin "coronas" appear, one overarching the egg and central cell and the other overarching the chalazal end of the synergid, as shown in Figure 3A. These actin coronas extend from approximately the middle of the synergid to the vicinity of the female target nuclei (Figure 3B). This is particularly interesting because myosin has been localized biochemically and immunochemically in the pollen tube (Tang et al., 1989; see Mascarenhas, 1993, this issue) and on the surface of pollen tube organelles and the generative cell of tobacco (Heslop-Harrison and Heslop-Harrison, 1989a, 1989b).

In the pollen tube, actin bundles in the cytoplasm interact with myosin on the surface of the organelles to maintain the polarity of cytoplasmic organelles during tube elongation (Pierson and Cresti, 1992). Such interactions are also believed to provide the principal mechanism for propelling the male cells through the tube and maintaining the association of the male reproductive cells with the vegetative nucleus (also, see Sanders and Lord, 1992). Actin is apparently absent within the male reproductive cells during their later development (Palevitz and Liu, 1992), but whether this is important to the transmission or fusion of the male cells is unclear.

Upon discharge of the pollen tube into the degenerate synergid, the male gametes are released from the cytoplasm of the tube and move into the chalazal end of the synergid. It is attractive to hypothesize that interactions between myosin on the surface of the male gametes and the actin coronas are

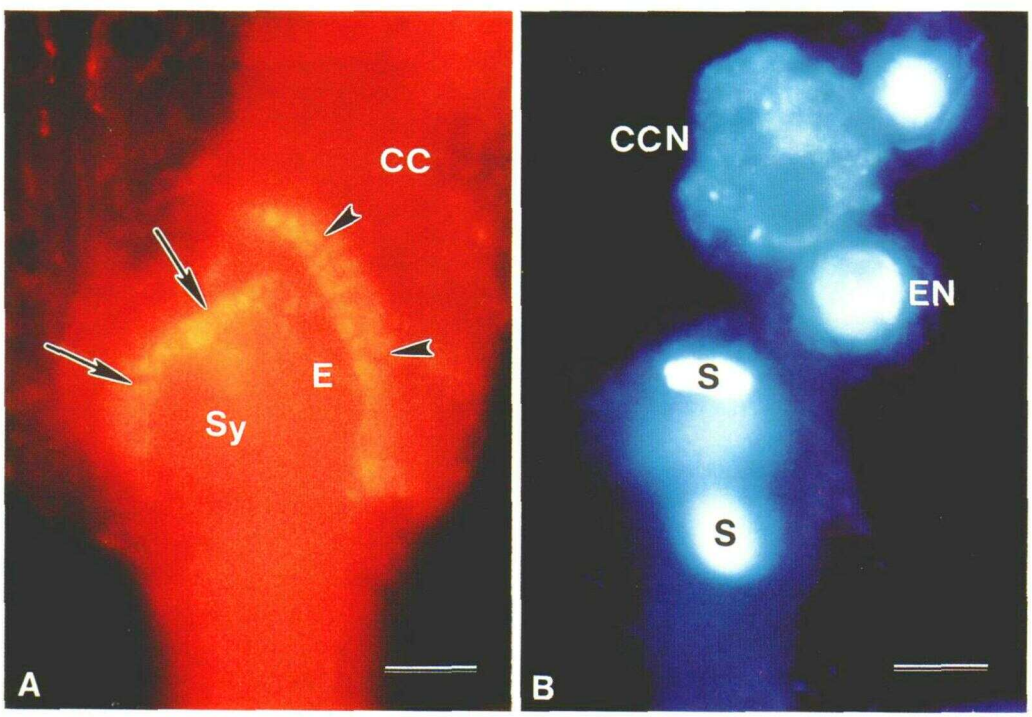

Figure 3. Localization of F-actin and Nuclear DNA in an Isolated Embryo Sac of Tobacco Soon after Fertilization.

(A) Brightly labeled actin, visualized by rhodamine–phalloidin staining, aggregates to form two "coronas" at the chalazal region of the degenerate synergid (Sy) (arrows) and at the interface between the egg (E) and central cell (CC) (arrowheads). Bar = 10 μm.

(B) Localization of sperm nuclei (S) in the chalazal region of the degenerate synergid, approaching nuclei of the egg (EN) and central cell (CCN). Nuclei are visualized with Hoechst 33258. Bar = 10 μm.

involved in sperm cell migration. The location of the coronas appears well adapted to such a function, but experimental support for this hypothesis is required. Using isolated embryo sacs, it may be possible to introduce sperm cells into the synergid, examine their interaction with the actin coronas, and experimentally alter conditions to test the requirements for fertilization.

Once the sperm cells reach the sites of fusion, their plasma membranes fuse with those of the female target cells (i.e., the egg and central cells). The energy required for plasma membrane fusion is sufficiently great that it seems likely that either specific fusigenic receptors are involved or that divalent cations, such as calcium, are needed to aid in the process of cellular fusion (see White, 1992). Unique plasma membrane conditions could also occur in the gametes, as is reported to occur in the plasma membranes of floral apices after induction (Crèvecoeur et al., 1992).

Once the cells fuse, the sperm nuclei are transmitted into the egg and central cells. The nuclei then migrate into alignment with their respective female target nuclei, and nuclear fusion begins. The outer membrane of the nuclear envelope is the first to fuse in premitotic fertilization, followed by the inner membranes. The widening of nuclear bridges linking the nucleoplasm of the male and female nuclei completes the fusion process (Jensen, 1974).

PREFERENTIAL FERTILIZATION

Because the MGU represents a naturally polarized assemblage—one sperm cell is consistently associated with the vegetative nucleus, whereas the other is connected to and trails behind the first—it is interesting that some species display sperm dimorphism in both their organization and cytoplasmic constitution, and some do not (Russell, 1991; Mogensen, 1992; see also Dumas and Mogensen, 1993, this issue). The presence of unequal quantities of heritable organelles in the sperm is responsible for what has been termed "cytoplasmic heterospermy," whereas differences in nuclear content (such as the proportion of B-chromosomes) cause "nuclear heterospermy" (Russell, 1985). Although cytoplasmic heterospermy is relatively common in flowering plants (see Russell, 1991), nuclear differences have been reported only in maize, in which B-chromosomes in the generative cell frequently do not segregate during generative cell mitosis, resulting in aneuploid sperm cells (Roman, 1948).

Cytoplasmic heterospermy usually consists of numerical differences in heritable organelles in the two sperm cells. Frequently, the sperm cell associated with the vegetative nucleus (S_{vn}) is larger and also contains more mitochondria. The most extreme form of dimorphism is that of *Plumbago*, in which the quantities of plastids and mitochondria are diametrically opposed between the two cells (Russell, 1984). The S_{vn} usually contains no plastids and over 200 mitochondria, whereas the other sperm cell (S_{ua}), which is linked to the S_{vn} but is unassociated with the vegetative nucleus, contains an average of 24 plastids and fewer than 50 mitochondria. To date, only members of the Plumbaginaceae have been reported to show this degree of plastid dimorphism (Corriveau and Coleman, 1988), but other species display dimorphism in mitochondrial content (Russell, 1991). Truly isomorphic sperm cells have also been reported, in which no statistically significant differences occur (Yu et al., 1992, and citations therein).

In sperm cells possessing strong patterns of dimorphism, it has been possible to trace the fate of each type of sperm cell and to determine whether fertilization is preferential. In *Plumbago*, the plastid-rich S_{ua} fuses with the egg 94% of the time (Russell, 1985) and, therefore, selectively transmits male plastids into the zygote. The ratio of male to female mitochondria, by contrast, remains approximately the same, 1:1000, in the egg and central cell; this occurs because differences in mitochondria in the two sperm cells nearly match differences in the mitochondrial content of the egg and central cell (Russell, 1987).

In maize sperm cells with nuclear differences, the sperm cell with extra B-chromosomes frequently fuses with the egg cell (up to 75% of the time; Roman, 1948; Carlson, 1969, 1986). The presence of supernumerary B-chromosomes alone seems to confer a selective advantage to the sperm cell because B-chromosomes of different origin express similar degrees of preferentiality. The presence of too many B-chromosomes negates this effect (Carlson, 1969). The maternal genotype can also alter the pattern of preferential fertilization because introducing the TB-9b B-chromosome into the egg cell eliminates preferentiality.

Maternal control of fertilization is also evident in a mutant line of barley, in which a normally fertilized endosperm is produced but the embryos are unfertilized (Mogensen, 1982). The sperm source appears to be unimportant, because any pollen results in unfertilized haploid embryos. The discrimination between the sperm cells seems to be maternally controlled and is apparent only under specific conditions. There are obvious benefits to using such mutants to understand how double fertilization is controlled. Even if preferential fertilization appears to be traceable only in systems with strong dimorphism, it provides evidence that discrimination between sperm cells in angiosperms may occur at the gametic level. The cause for discrimination is unknown; immunocytochemical, biochemical, and freeze-fracture studies of sperm cells may provide insight into the underlying mechanisms of preferential gametic fusion.

CYTOPLASMIC INVOLVEMENT DURING FERTILIZATION

The transmission of heritable cytoplasmic organelles during double fertilization remains an area of controversy and apparent variability among angiosperm species (see Russell et al.,

1990). The male cytoplasm is clearly transmitted in some plants (Russell, 1983) but not in others (Jensen and Fisher, 1968; Mogensen, 1988). A number of mechanisms that would reduce the likelihood of male cytoplasmic transmission prior to sperm cell deposition have been proposed: (1) organellar diminution through the pinching off of cellular processes (see Mogensen, 1992) or production of enucleated cytoplasmic bodies (Yu and Russell, 1992; Yu et al., 1992); (2) alteration of organelles (Vaughn et al., 1980); (3) modification of organellar DNA through molecular means (Day and Ellis, 1984), including specific nucleases (e.g., nuclease C; Nakamura et al., 1992); or (4) low ratios of paternal to maternal organelles (Russell, 1987), resulting in a high probability of paternal organelle extinction (Birky, 1983) or in levels of expression that are below the threshold for detection (Milligan, 1992). Just prior to fusion, the sperm cells may shed their cytoplasm outside the egg and central cells, as in cotton (Jensen and Fisher, 1968), or just outside of the egg cell, as in barley (Mogensen, 1988).

The pattern of plastid and mitochondrial inheritance follows one of three possible patterns for each organelle type: (1) uniparental maternal, (2) biparental, or (3) uniparental paternal. Despite these numerous possibilities, both organelles are inherited predominantly in a uniparental maternal pattern. A significant minority of flowering plants displays inheritance of some paternal plastids, but the fate of paternal mitochondria is less clear (Russell, 1992). Paternal mitochondrial RNA is inherited in alfalfa (Fairbanks et al., 1988), and paternal mitochondrial DNA has been detected in *Populus* (Rajora et al., 1992). In *Brassica napus*, paternal mitochondria may predominate in the embryo (Erickson and Kemble, 1990). As molecular techniques become more sensitive, the number of plants known to transmit at least some male cytoplasmic DNA will increase; occasional leakage of male organelles may be one reason that mechanisms to diminish the quantity of male organelles may be redundant.

Uniparental paternal inheritance of organelles has recently been reported in alfalfa and in some species of *Daucus*. In these plants, the paternal plastids are inherited in the embryo, and maternal plastids are undetected (Fairbanks et al., 1988; Schumann and Hancock, 1989; Boblenz et al., 1990; Masoud et al., 1990).

EGG ACTIVATION, ZYGOTE FORMATION, AND ZYGOTE DIVISION

Although egg activation occupies a significant literature in animal cell biology (e.g., Alberts et al., 1989), the changes that occur in the angiosperm egg as a result of fertilization are poorly described. In most animal egg cells, fusion with a sperm cell triggers a fast block to further fusions by causing a rapid depolarization of the membrane (Jaffe and Cross, 1986). After a latency period of less than a minute, numerous cortical granules fuse with the plasma membrane, producing a 1.7-fold increase in membrane capacitance and creating an impenetrable fertilization membrane around the fertilized egg cell while membrane conditions return to normal. These events occur in many animals and in the alga *Fucus*. Possibly, the same form of hyperpolarization occurs in the membrane of the egg cell in angiosperms, also preventing polyspermy. Cortical granules are, however, not formed, and there are no reports of distinct fertilization membranes in angiosperms.

Fertilization in animals is also accompanied by the opening of ion channels, and changes occur in the state of free calcium. In angiosperms, dramatic changes also occur in the state of calcium, but these occur within the synergid. High concentrations of calcium are released during the degeneration of the synergid and the collapse of the vacuole (Chaubal and Reger, 1992a, 1992b). Chlorotetracycline, which preferentially stains membrane-bound calcium, labels the synergid intensely after vacuolar breakdown, suggesting that significant quantities of calcium are sequestered in membranes of the degenerated synergid (Huang and Russell, 1992b). The presence of high calcium concentrations between the cells of the FGU (Chaubal and Reger, 1990, 1992a) may influence the immediate environment of fusion in the degenerated synergid. Materials discharged from the pollen tube during the process of gamete release may further condition the environment for gametic fusion. The involvement of calcium in so many facets of cellular behavior and membrane fusion in cells (e.g., Alberts et al., 1989) suggests that the high concentrations of calcium released during synergid degeneration in angiosperms, even though intercellular, may significantly affect fertilization and postfertilization events.

The ultrastructural organization of the fertilized egg cell frequently displays an active appearance that is unlike that before fertilization occurs (Jensen, 1974; van Went and Willemse, 1984; Mansfield and Briarty, 1991, Mansfield et al., 1991). This extends to all of the major classes of organelles and includes the closure of the cell wall around the zygote. The area around the entry point of the pollen tube into the embryo sac is also a site of intense cell wall formation.

A major cytoskeletal event indicating the restoration of the sporophytic program is the occurrence of a PPB of microtubules prior to mitosis of the zygote. In typical somatic cells, the PPB predicts the location of the metaphase plate and future cell plate formation (Gunning and Hardham, 1982). The normal pattern of PPB formation returns as the PPB coalesces over a relatively long time period (Webb and Gunning, 1991). Thereafter, subsequent mitosis in the embryo always involves a PPB. Why the PPB is absent in reproductive cells and the endosperm is not yet understood; the reason for the restoration of the PPB in the embryo also remains to be determined.

CONCLUSION AND PROSPECTS

The egg cells of flowering plants have been the subject of numerous studies (for review, see Huang and Russell, 1992a). The unfertilized egg cell appears, from its ultrastructural and

cytoskeletal characteristics, to be an unspecialized parenchyma cell. Whether only specific domains of the egg cell are receptive for fusion, and the precise roles of surrounding cells in controlling the passage of sperm cells into the egg and central cell, are some of the questions that will need to be resolved in the future. The egg cell is an example of a naturally recombinant partial protoplast that is potentially adapted to receive foreign genetic material and stably pass its genetic complement to the succeeding generation. The use of gametes as a potential source for haploid cells may present significant advantages in forming defined cell lineages and in genetic recombination, especially if the genome proves to be more stable in gametes than in other cell sources. Additional possibilities include the use of eggs as a haploid germplasm and as a source for haploid calli in general.

The ultimate objective of much of the current research into the organization and physiology of egg cells is to understand how double fertilization in flowering plants operates, the limits of variability in fertilization mechanisms, and how the organization of the egg cell is reflected in the zygote and embryo. The establishment of a model system for in vitro fertilization that allows the opportunity to describe the physiology of flowering plant gametes directly presents significant opportunities that remain to be exploited in this area of research.

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