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Exclusion of male mitochondria and plastids during syngamy in barley as a basis for maternal inheritance

(embryo sac/fertilization/quantitative ultrastructure/sperm cells)

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ABSTRACT **It is known from genetic analyses that maternal inheritance of cytoplasmic organelles is the rule among plants and animals. Although recognized as one of several possible mechanisms for strictly maternal cytoplasmic inheritance, exclusion of sperm cytoplasm at the time of gametic fusion has remained poorly documented for the flowering plants. In the present investigation, enucleated, cytoplasmic bodies approximately the size of intact, prefusion sperm cells have been observed within degenerated synergids and adjacent to recently fertilized egg cells. A complete series of ultrathin sections (68 sections) through such a cytoplasmic body revealed 59 mitochondria, 3 plastids, 7 dictyosomes, and a large vacuole with no limiting membrane. This structure is interpreted as the entire male cytoplasm that was left outside the egg during fusion between egg and sperm. The observation of only one cytoplasmic body per embryo sac may indicate a preliminary fusion between sperm cells or, more likely, the existence of a fundamentally different mechanism of fertilization between the second sperm and the central cell.**

With few exceptions, extranuclear organellar DNA is inherited strictly maternally in plants and animals (1–5). In flowering plants, several mechanisms have been considered responsible for the uniparental inheritance of plastids and mitochondria: (*i*) exclusion of the organelles from the generative cell or one sperm cell during the first or second pollen mitosis, respectively (6, 7); (*ii*) deletions in plastid DNA, degeneration of organelles, or elimination of organelle-containing cytoplasm during microspore, generative, or sperm cell maturation (8–11); (*iii*) organelle exclusion during gamete fusion (4, 12, 13); (*iv*) degeneration, lack of replication, or compartmentalization into suspensor cells after zygote formation (14–16).

Of these possible mechanisms, exclusion of male cytoplasm during syngamy has been poorly documented and, thus, is the most controversial (4, 6, 17). Structural evidence for such a phenomenon has been reported for cotton (18), in which two membrane-bound, enucleated cytoplasmic bodies were found within the degenerated synergid shortly after fertilization. Although the investigators recognized that these structures may be of sperm origin, it is not possible to assess what proportion of the sperm cytoplasm may be represented by these bodies—i.e., no quantitative data were given and only one mitochondrion within the presumed male cytoplasm was illustrated. Wilms (12) proposed a similar exclusion mechanism for the male cytoplasm of spinach; however, his only documentation was that of a binucleate cell (interpreted as fused sperm cells), containing sparse cytoplasm, located within the intercellular space between the egg and central cell. Hagemann (6) and Hagemann and Shröder (4) propose for several grass species that the male cytoplasm is "stripped" off from the male nucleus during the fertilization process, since plastids and mitochondria are present within the sperm cells of wheat and other cereals, yet cytoplasmic inheritance is strictly maternal.

Based upon the presence of what appeared to be male cytoplasmic sheaths just outside recently fertilized eggs of barley, I reported (13) that exclusion of paternal cytoplasm at syngamy likely occurs in this plant; however, documentation in this case consisted only of light microscopic data. In the present study, serial thick sections and serial ultrathin sections from reembedded thick sections were used for a detailed ultrastructural analysis and quantitation of one of these enucleated, cytoplasmic bodies located within the degenerated synergid and adjacent to the recently fertilized egg cell of barley. The results indicate that the organelle complement of this body accounts for that expected within prefusion sperm cells of this plant.

MATERIALS AND METHODS

Barley plants (*Hordeum vulgare* L.) used for this study were either glasshouse-grown at the Carlsberg Plant Breeding Station (Hyldagergaard, Denmark), or field-grown at the United States Department of Agriculture–University of Arizona plant breeding station in Tucson, Arizona. Spikes were emasculated and bagged 2 or 3 days before hand pollinations were performed. Ovaries were trimmed under moist conditions and initially fixed in 4% glutaraldehyde in Sorensen's phosphate buffer (pH 7.4). Collections were made at 3- to 5-min intervals during a 90-min period after pollination. After 6 hr at room temperature the tissue was rinsed in phosphate buffer, postfixed in 2% osmium tetroxide (in same buffer) for 2 hr at room temperature, rinsed in distilled water, dehydrated in an ethanol/acetone series, and embedded in Spurr's resin (11, 13).

Serial thick sections (about 3 μm) were cut with a glass knife and observed with phase-contrast optics. Selected thick sections were then reembedded (19), serially ultrathin sectioned (about 75 nm) with a diamond knife, stained with uranyl acetate and lead citrate, and observed with transmission electron microscopy.

Ultrastructural information on the embryo sac containing the enucleated, cytoplasmic body of the present study was obtained from 4 reembedded thick sections. The cytoplasmic structure was present within 3 thick sections and 68 ultrathin sections. The drawing of Fig. 1 is a composite based upon projections of photographic negatives taken from the 4 thick sections.

RESULTS

After their discharge from the pollen tube, the two sperm cells become positioned near the chalazal end of the degenerated synergid (13, 20). Often, the sperms are found beyond the degenerated synergid cytoplasm and within the intercellular space between the egg and central cell (Fig. 2; ref. 13).

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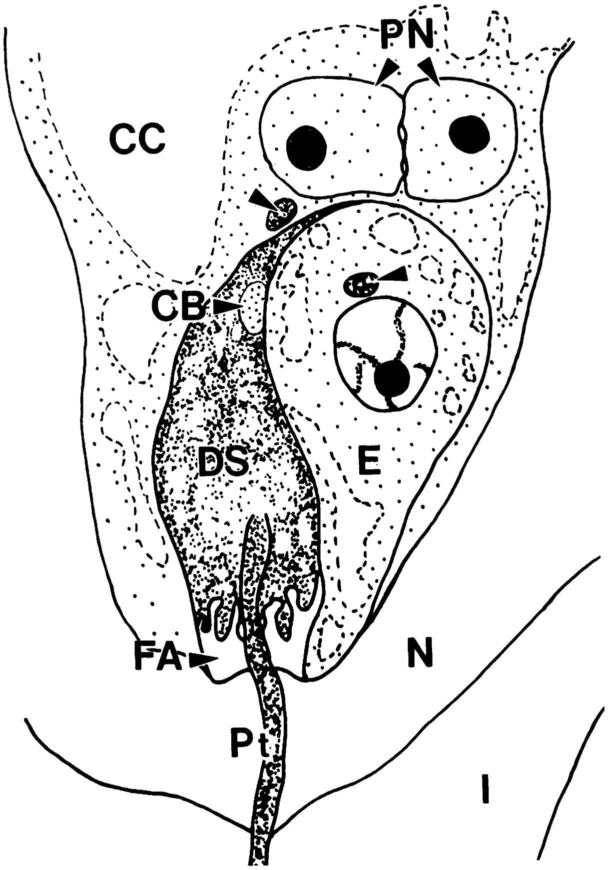


FIG. 1. Composite drawing based upon four serial thick sections from the same embryo sac showing an early postsyngamy stage. The pollen tube (Pt) has entered the degenerated synergid (DS) and discharged the two sperm cells. One sperm nucleus (unlabeled arrowhead) is within the egg cell (E), near the egg nucleus. The other sperm nucleus (unlabeled arrowhead) is within the central cell (CC) near the polar nuclei (PN), which have begun to fuse. Within the degenerated synergid and adjacent to the egg cell is a cytoplasmic body (CB), which is interpreted to be sperm cytoplasm that was excluded at the time of fusion between egg and sperm. FA, filiform apparatus; I, inner integument; N, nucellus; dashed lines outline vacuoles.

Although the sperms are connected to each other within the pollen tube (21), they have not been observed to be in contact within the embryo sac (present study; ref. 13).

Several embryo sacs show what appears at the light microscope level to be an enucleated, cytoplasmic body just outside the recently fertilized egg (present study; ref. 13). One such early postsyngamy stage is diagramed in Fig. 1; the embryo sac on which this drawing was based is the same as that from which the following data were obtained. Note that one male nucleus is present within the egg cell (Figs. 1 and 3) and the other is within the central cell (Figs. 1 and 4). Neither sperm nucleus has yet reached the female nucleus with which it was destined to fuse (Figs. 1, 3, and 4). Located within the degenerating synergid and at the edge of the egg slightly chalazal to midlevel is an enucleated cytoplasmic body (Figs. 1, 5, and 6), which is interpreted to be sperm cytoplasm excluded at the time of fusion between sperm and egg. Its position is near the level of the sperm nucleus within the egg cell (Fig. 1). Analysis of complete serial ultrathin sections through this structure indicates that it contains 59 mitochondria, 3 plastids, 7 dictyosomes, and a rather large nonmembrane-bound vacuolate area at the side toward the egg cell. A plasma membrane is present around much of the cytoplasm, but there are large areas of discontinuities on the sides away from the egg cell and adjacent to the degenerated synergid cytoplasm. Two plasma membranes are evident at a few places along the side where the cytoplasmic structure is adjacent to the egg (Fig. 5). At no point is there an opening between the cytoplasmic body and the egg cell. The cytoplasmic body measures 6.7 μm × 3.8 μm; the vacuolate area is 3.7 μm × 3.5 μm. The sperm nucleus within the egg cell of this embryo sac is approximately 7.0 μm × 4.0 μm. Intact prefusion sperm cells within barley embryo sacs measure from 6.0 μm to 8.0 μm in length and from 3.5 μm to 5.9 μm in width.

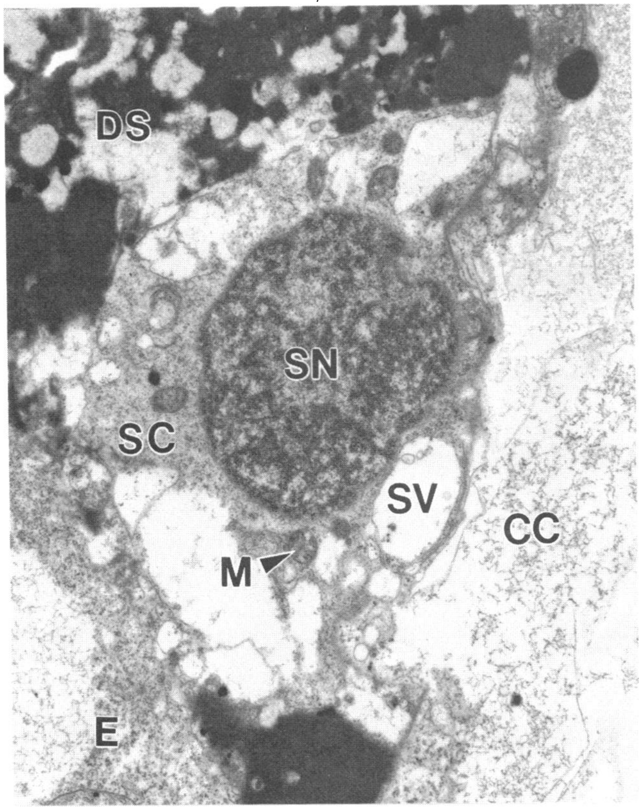


FIG. 2. Intact, prefusion sperm cell (SC) containing mitochondria (M) and a nucleus (SN), located between the egg (E) and central cell (CC). DS, degenerated synergid; SV, sperm vacuole. (×10,600.)

DISCUSSION

Several factors support the interpretation that the enucleated, cytoplasmic body of the present study represents sperm cell cytoplasm left behind after the male nucleus entered the egg. (*i*) Many of these structures have been observed at the light microscope level within recently fertilized embryo sacs; none has been seen prior to fertilization. (*ii*) The size and shape of the cytoplasmic body are roughly similar to that of intact sperm cells within the embryo sac. The slightly smaller size of the cytoplasmic body is not unexpected, since the nucleus is no longer present. (*iii*) The cytoplasmic structure contains organelles known to be present within barley sperm cells (11, 13, 22, 23). (*iv*) The cytoplasmic body contains a nonmembrane-bound, vacuolated area devoid of organelles, which would be expected in such a body from which its nucleus had exited. That this vacuolate area is somewhat smaller than the sperm nucleus may be due to some invasion of surrounding cytoplasm into the space previously occupied by the nucleus. It is also possible that the sperm nucleus enlarges after entering the egg cell. (*v*) The location of the cytoplasmic body within the degenerated synergid and next to the egg cell places it in the vicinity of the sperm nucleus within the egg cell. (*vi*) Alternative origins

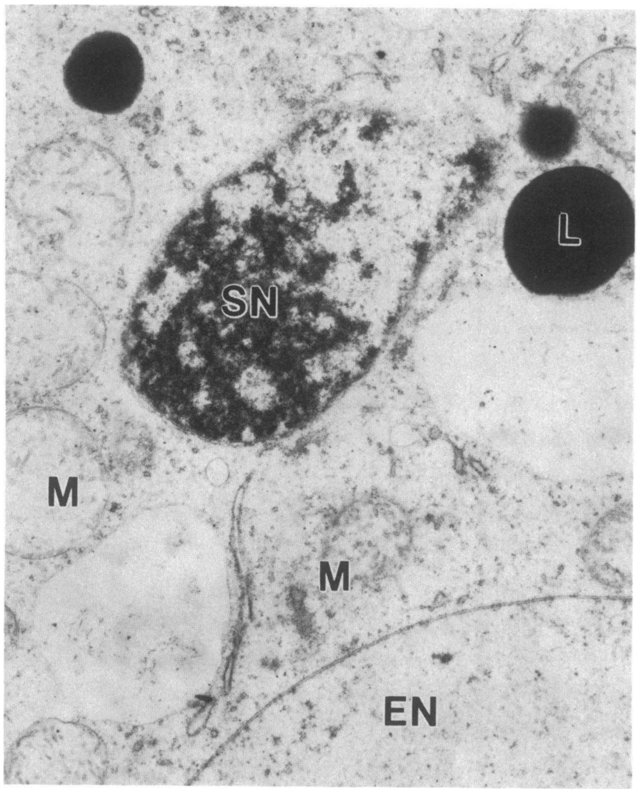


FIG. 3. Sperm nucleus (SN) within the egg cell, near the egg nucleus (EN). L, lipid body; M, mitochondria. From the embryo sac of Fig. 1. (×10,600.)

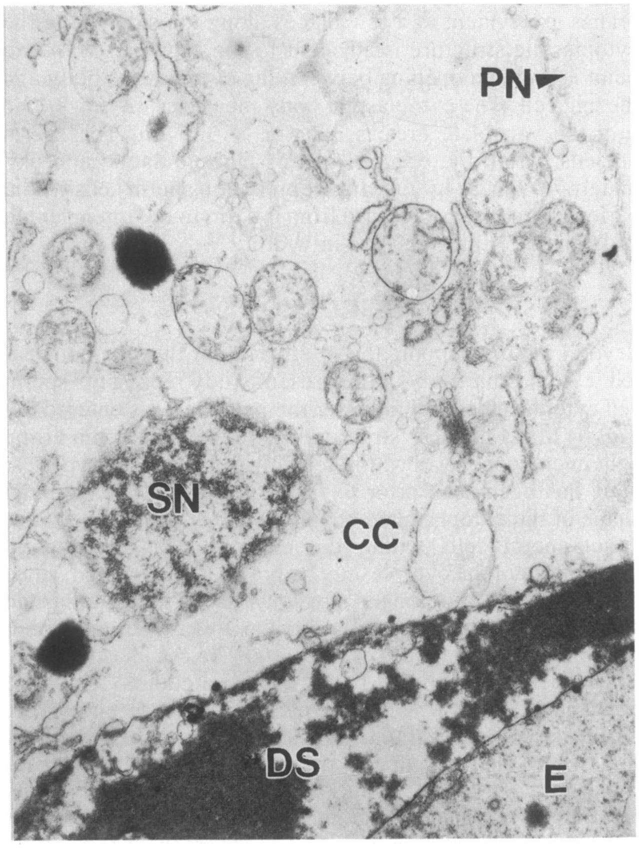


FIG. 4. Portion of the sperm nucleus (SN) within the central cell (CC), near one polar nucleus (PN). E, egg cell; DS, degenerated synergid. From the embryo sac of Fig. 1. (×10,600.)

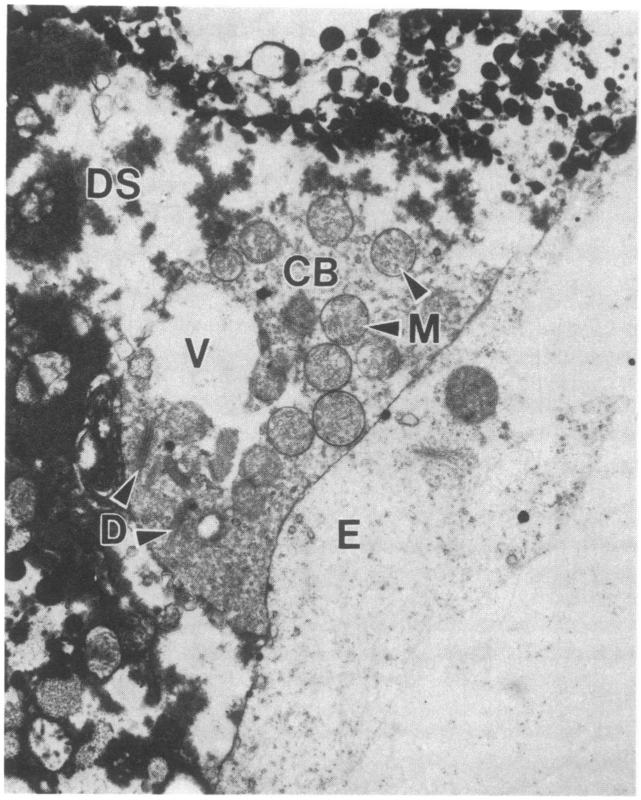


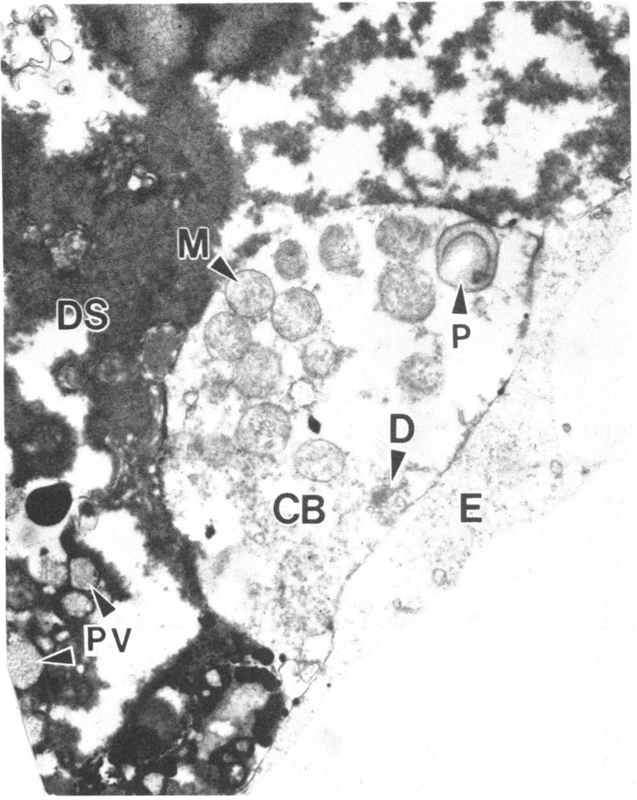
FIG. 5. Cytoplasmic body (CB) within the chalazal end of the degenerated synergid (DS) showing mitochondria (M), dictyosomes (D), and a vacuole (V). Serial sections show that this structure is enucleated and that the vacuole extends to, and is largest near, the side next to the egg (E). From the embryo sac of Fig. 1. (×10,600.)

for this structure are difficult to explain, since the cytoplasm of both synergids and that of the pollen tube have already degenerated. (*vii*) Plastid inheritance in barley is strictly maternal (2).

Assuming that this interpretation is correct, results of the present investigation strongly suggest that a mechanism for major, if not total, exclusion of paternal cytoplasm is operating at the time of gamete fusion in barley. Although data from somatic hybridization studies indicate that it may not matter which of the two donor cytoplasmic genomes is eliminated (24, 25), the mechanism proposed here for barley ensures that only maternal cytoplasm is inherited. This mechanism may be important during sexual reproduction for the nontransmission of genetically altered male organellar genomes, such as large deletions in chloroplast DNA known to occur in microspores of wheat and barley (8, 26).

The finding of only a single cytoplasmic body outside the recently fertilized egg cell could indicate that the sperm cell fusing with the central cell transmits its cytoplasm, which could subsequently be incorporated into the developing endosperm. Alternatively, the presence of a single male cytoplasmic body could mean that, similar to the interpretation for spinach (12), cytoplasms of the two sperms have fused. The occurrence of at least 59 mitochondria within this body lends support to this conclusion, since the mean number of mitochondria per sperm cell at anthesis in barley is 31 (11). However, it is very possible that sperm cell mitochondrial proliferation occurs after pollination. This would not be surprising in light of the postpollination sperm morphogenesis known to occur in barley (21).

Information has not yet been obtained in barley that would offer insight into the precise mechanism resulting in the exclusion of male cytoplasm during gamete fusion. That gamete fusion begins with the coalescence of participant



proposed here have been described by Jensen and Fisher

24. Shiller, R., Herrmann, R. G. & Melchers, G. (1982) *Mol. Gen.*

cells of barley (13).

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