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Embryology of barley. IV. Ultrastructure of the antipodal cells of *Hordeum vulgare* L. cv. Bomi before and after fertilization of the egg cell

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Abstract The antipodal cells have been the stepchildren in most investigations of the female gametophyte. In *Hordeum vulgare* cv. Bomi, three antipodal cells are originally developed chalazally but because of differential growth of the embryo sac they soon become laterally situated and their number increases to 35–50 cells and the shape, size and structure of the cells change in the time before as well as after fertilization. The cells persist until about 60–70 h after pollination. At that time, the embryo consists of about 12–15 cells and a cellularization of the nuclear endosperm has started peripherally. The size of nuclei, and especially nucleoli, in the antipodal cells increases tremendously in the investigated period and the amounts of organelles also change. The walls of antipodals are diversified depending on which cells they are separating, and wall invaginations are developed especially between antipodal cells and surrounding nucellar cells in the placental region. The mitochondria appear in various shapes in section view, very often as cups or dumbbells with a rim in the ends containing cristae and a thin cristae-free base. These bases are sometimes stretched out as thin parts and at last a simple parting occurs. Binary fissions of the plastids happen especially in the hours before and just after egg fertilization. ER is extraordinarily well developed in the whole period of investigation and many ribosomes are attached to the membranes. Dictyosomes form numerous vesicles, especially in the antipodals near the nucellar cells in the placental region. These ultrastructural details support the opinion that antipodal cells may play an important role in the embryo sac and are able to be responsible for the supply of nutrition for the whole gametophyte and take part in the supply of nutrition during embryo formation.

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Functions · *Hordeum vulgare* · Gramineae

Introduction

The purpose of this paper is to describe the structure and development of antipodal cells in *Hordeum vulgare* L. cv. Bomi in order to determine the ultrastructural changes occurring in the interval of time from before pollination to 4–5 days after fertilization.

Earlier papers in this series (Engell 1988, 1989), describe the time course and analyse controlled fertilization and early embryo formation, as well as investigate the synergids before and after fertilization. Antipodal cells have received previously little attention (Engell 1991a).

Generally, antipodal cells have been the stepchildren in investigations of female gametophytes. They are of course treated in books and reviews (Johri 1984; Kapil and Bhatnagar 1976, 1981; Vijayaraghavan et al. 1988; Chapman 1985; etc.), but in light microscopy studies, there have in general only been countings of antipodal cells and determinations of their life span.

In a few cases among grasses, the antipodal cells have been subjected to further investigations: Gramineae in general (Chikkannaiah and Mahalingappa 1975); *Phleum* (Joachimiak 1981); *Triticum* (Hoshikawa 1960); *Hordeum* × *Secale* (Brink and Cooper 1944); Cooper and Brink (1944); Thompson and Johnston (1945).

The antipodals are also featured in a few ultrastructural investigations outside the grasses, for example, in *Aconitum* (Bohdanowicz and Turala-Szybowska 1985, 1987) and in *Gasteria* (Willemse 1981; Willemse and Kapil 1981), where the works cited feature analyses and discussions of cells and their functions.

At the ultrastructural and histochemical level, there are some investigations of the embryo sacs of grasses, particularly studies of *Zea* (Diboll and Larson 1966; Diboll 1968; Russell 1978; Lammeren 1987), *Stipa* (Maze and Lin 1975), *Triticum* (You and Jensen 1985),

Hordeum (Cass and Jensen 1970; Cass et al. 1985; Cass and Peteya 1986), and *Paspalum* (Yu and Chao 1979). None of these investigations was made for study of the antipodal cells themselves but for the whole embryo sac and its contents.

Materials and methods

The cells of the embryo sac have been studied from 48 h before (h.b.p.) a controlled pollination was carried out and in the time when total degeneration of the antipodal cells took place about 70–80 h after pollination (h.a.p.).

Details on the growth conditions of the plants and time schedule for sampling of specimens for serial analysis of the embryo sac were described earlier (see Engell 1989 for LM- and Engell 1988 for TEM-preparations).

Results

In *Hordeum vulgare* three antipodal cells are originally developed chalazally (Cass and Peteya 1986). The differential growth of the nucellus and/or the gametophyte, however, soon causes the cells to become laterally situated adjacent to the side wall facing the placental region in the anatropous, nearly tenuinucellate and bitegmic ovule (Fig. 1). Forty h.b.p. there are already several cells in the lateral position. The numbers range from 35 to 50 in the next 5–6 days. Divisions in the antipodal cells have not seen; all the cells are permanently uninucleate but dimensions of the nuclei increase.

Measurements at different ages of the ovule, the embryo sac, and the whole antipodal complex show changes in dimensions from the time before it is possible to make a controlled pollination through the next 3–4 days after pollination (Table 1). The growth of all structures, the antipodal tissue included, begins before and continues after fertilization.

The shape as well as the structure of the individual cell also changes. The antipodal cells are nearly rectangular (Fig. 2A) or slightly pointed toward the placental region as long as the embryo sac and its cells are immature. The antipodal cells stand as a fan from the placental region toward the middle of the embryo sac (Fig. 2B). This shape is nearly preserved until 30 h.a.p., but about this time the cells undergo a considerable stretching in the longitudinal direction of the embryo sac (ES) com-

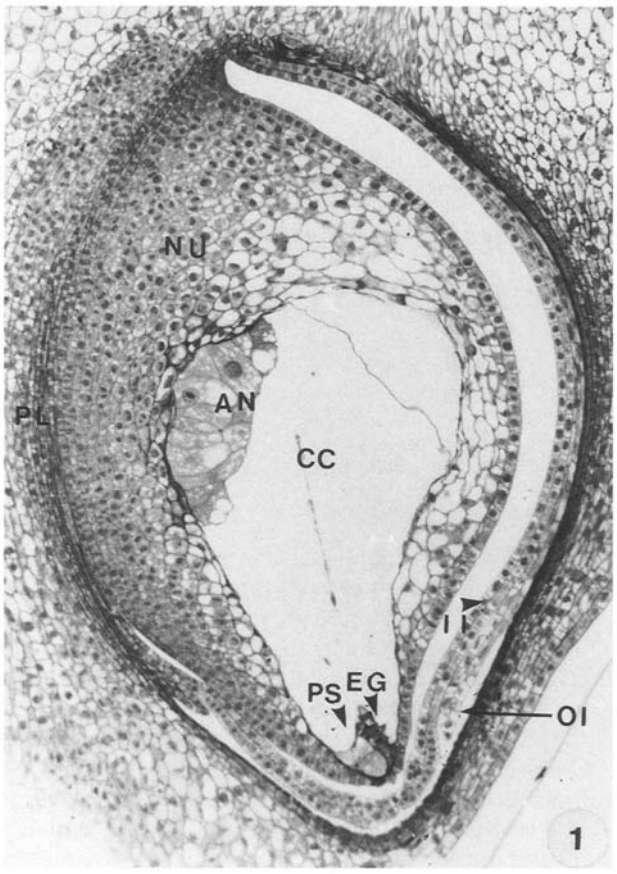


Fig. 1 Anatropous, bitegmic ovule with the antipodal cells laterally placed, adjacent to the placental region, 14 h before the controlled pollination (14 h.b.p.). ×140. *Abbreviations* (all figures): *AA* antipodal cell, *CC* central cell, *CW* cell wall, *D* dictyosome, *EG* egg cell, *EN* endosperm, *ER* endoplasmic reticulum, *II* inner integument, *LO* lomasome, *M* mitochondrion, *NC* nuclear invagination, *NU* nucellus, *OI* outer integument, *P* plastid, *PC* plastid constriction, *PD* plasmodesma, *PL* placental region, *PO* polysome, *PS* persisting synergid, *V* vacuole

Table 1 Dimensions (×10 μm) of the ovule, embryo sac, and antipodal complex in the period from 40 h before to 50 h after a controlled pollination. Length and depth are the dimensions of the contact area between the antipodal complex and the placental region, while width is the dimension of the complex in the lumen of the embryo sac. Mean value was calculated from at least five measurements; *stds* sample standard deviation

Time	Ovule		Embryo sac		Antipodal complex		
Hours after pollination	Length Mean ± stds	Width Mean ± stds	Length Mean ± stds	Width Mean ± stds	Length Mean ± stds	Width Mean ± stds	Depth Mean ± stds
−40	64 ± 6	45 ± 1	34 ± 2	17 ± 1	17.3 ± 0.5	5.8 ± 0.1	11.1 ± 0.1
0	66 ± 4	43 ± 4	35 ± 3	24 ± 2	26.5 ± 1.4	10.3 ± 1.2	14.4 ± 0.2
6	71 ± 4	49 ± 2	46 ± 1	28 ± 5	18.6 ± 0.1	9.7 ± 0.7	17.8 ± 0.3
13	81 ± 7	59 ± 4	57 ± 5	37 ± 3	28.6 ± 0.5	5.5 ± 0.2	16.5 ± 0.1
20	86 ± 2	57 ± 2	59 ± 2	40 ± 3	34.2 ± 0.1	10.5 ± 0.3	29.0 ± 0.6
30	117 ± 5	53 ± 4	89 ± 4	27 ± 2	33.5 ± 1.1	13.5 ± 0.3	28.8 ± 0.1
50	181 ± 26	77 ± 13	145 ± 17	55 ± 4	57.1 ± 5.3	14.0 ± 1.7	24.2 ± 0.3

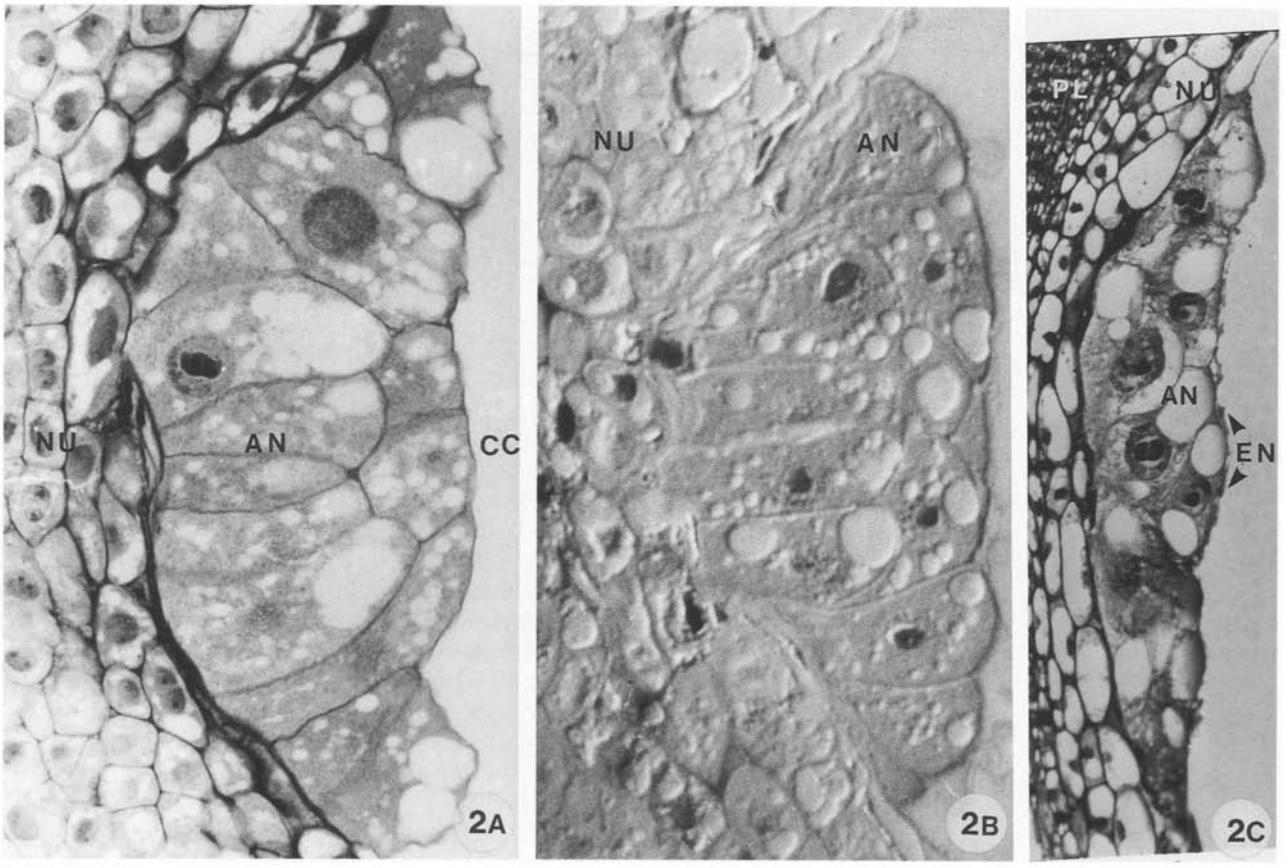


Fig. 2A–C Longitudinal sections of the antipodal cell complex fixed at different times. **A** The cells have small vacuoles, especially those near to the central cell (14 h.b.p.). ×562. **B** Fanshaped antipodal complex (44 min a.p.). ×675. **C** The antipodal cells with few, large vacuoles (59 h.a.p.). ×225. **A,C** light micrographs; **B** interference contrast micrograph

Table 2 Dimensions (×10 μm) of individual cells in the embryo sac at different times. The egg cell continues as the zygotic cell and its derivatives after pollination (0 h.a.p.). Both synergids disappear before 30 h.a.p. Mean value was calculated from at least five measurements; *stds* sample standard deviation

Time	Egg cell		Synergid		Antipodal cell	
Hours after pollination	Length Mean ± stds	Width Mean ± stds	Length Mean ± stds	Width Mean ± stds	Length Mean ± stds	Width Mean ± stds
−40	4.9 ± 0.2	3.4 ± 0.3	4.1 ± 0.6	2.2 ± 0.5	4.5 ± 0.2	2.3 ± 0.3
0	6.0 ± 0.5	3.5 ± 0.3	5.3 ± 0.5	3.3 ± 0.4	5.2 ± 0.2	3.2 ± 0.3
6	6.1 ± 0.5	3.5 ± 0.4	5.6 ± 0.3	3.0 ± 0.2	3.6 ± 0.3	4.8 ± 0.5
13	7.0 ± 0.4	4.2 ± 0.2	5.5 ± 0.4	2.9 ± 0.4	3.7 ± 0.3	6.1 ± 0.6
20	8.0 ± 0.4	3.8 ± 0.3	6.0 ± 0.5	2.1 ± 0.2	3.6 ± 0.3	5.9 ± 0.4
30	8.0 ± 0.3	4.6 ± 0.4			8.0 ± 0.8	5.3 ± 0.5
50	8.4 ± 0.4	4.8 ± 0.3			11.2 ± 0.6	6.0 ± 0.6

bined with a little reduction in the transverse direction (the width of the cells) toward the middle of the ES. In this way, the area of the antipodal cells facing the placental region increases (Fig. 2 C).

A comparison among all cells in the embryo sac shows that antipodal cells are the ones with the greatest changes in dimensions (Table 2). The embryo consists at 50 h.a.p. of 7–8 cells, but the embryonal cells decrease in dimensions for each division in this period (Engell 1989).

Elongation of the individual antipodal cell is a result of vacuolation of the cytoplasm, together with an increase in dimensions of the nucleus and nucleolus (Fig. 3 and Table 3). From originally being nuclei of nearly the same size as nuclei in the other cells of the embryo sac, the antipodal nuclei grow larger. They enlarge slightly

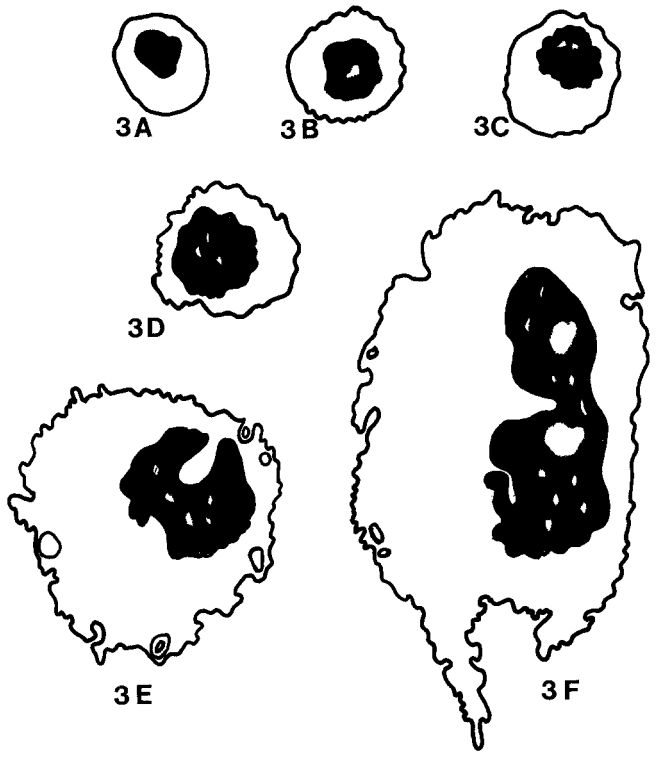


Fig. 3A–F Drawings of nuclei of antipodal cells fixed at different times: **A** 48 h.b.p., **B** 40 h.b.p., **C** 6 h.a.p., **D** 18 h.a.p., **E** 43 h.a.p., **F** 50.5 h.a.p. × 340

Table 3 The average dimensions (× 10 μm) of nuclei and nucleoli of antipodal cells in the period from 40 h.b.p. to 50 h.a.p. Mean value was calculated from five or six measurements; *stds* sample standard deviation

Time	Antipodal nucleus		Antipodal nucleolus	
Hours after pollination	Length Mean ± stds	Width Mean ± stds	Length Mean ± stds	Width Mean ± stds
−40	1.5 ± 0.2	1.3 ± 0.1	0.7 ± 0.06	0.5 ± 0.05
0	1.6 ± 0.1	1.6 ± 0.2	0.7 ± 0.08	0.6 ± 0.08
6	1.8 ± 0.2	1.6 ± 0.2	0.7 ± 0.08	0.7 ± 0.08
13	1.9 ± 0.2	1.5 ± 0.2	0.8 ± 0.08	0.6 ± 0.07
20	2.3 ± 0.3	1.9 ± 0.2	1.1 ± 0.13	1.1 ± 0.13
30	2.3 ± 0.3	2.1 ± 0.2	0.9 ± 0.09	0.8 ± 0.09
50	3.7 ± 0.4	3.2 ± 0.3	1.4 ± 0.14	1.2 ± 0.14

in the hours before and just after fertilization, but by 18–20 h.a.p. they are double in size and, about 50 h.a.p., their dimensions may be more than five times the size of the original nuclei (Fig. 3) but, on average, the nuclei are two and a half times the originals. The nucleoli grow even more. In some cases, their dimensions may be doubled about 20 h.a.p.; in other cases, an eight-fold magnification of dimensions was observed at 50 h.a.p.(Fig. 3).

Together with these enlargements, the shape of the nuclei also changes. The youngest nucleus, about 48 h.b.p., is nearly spherical with the heterochromatin spread out in the nucleoplasm, but soon the circumference of the nucleus will be slightly irregular and, in the next days, this becomes increasingly evident. Small intrusions of the nuclear membrane are formed later in the period and, about 43–50 h.a.p., invaginations into the nucleus form and other organelles are found inside these (Fig. 3E, 4C). Furthermore, the overall shape of the nucleus is changed to slightly elongated and an adaption to the surroundings (walls, vacuoles, etc.) takes place (Fig. 3F).

The heterochromatin is gathered in compact clusters (Fig. 4C, 9A) and, therefore, when the nuclei increase, the amount of chromatin makes up a lesser part of the whole nucleus. Zones nearly free of chromatin are left around the nucleoli, which, over time, are roundish and compact with a slightly diffuse circumference, with small, light areas inside; but, about a day after pollination the nucleoli increase and become more vacuolated and their shapes becomes more irregular, elongated and lobed. Sometimes they are extraordinarily large and nearly partitioned into two areas with large and small vacuoles intermingled (Fig. 3E,F).

The cells seem in the earliest studied stages to possess a high cytoplasmic density (Fig. 4A), despite the presence of many small vacuoles especially gathered in the end of the antipodal cells facing the central cell (Fig. 2A).

About the time of fertilization [45 min after pollination, m.a.p. (Engell 1989)], fusions of some of the small vacuoles occur, but these are still mostly placed near to the central cell (Fig. 2B) and such a position is kept throughout the first day after pollination (and fertilization). The largest vacuoles are continuously placed in the antipodal cells just inside the walls against the newly started nuclear endosperm (the development starts at 24 h.a.p.), while other, medium-sized vacuoles are found throughout in the cytoplasm.

The cells begin to extend longitudinally about 25–30 h.a.p. and the vacuoles fuse to very large ones (one or two) in each end of the cell (Fig. 2C). The cells will by this elongation obtain great surface area for mutual contact both with the placental nucellar region and the developing nuclear endosperm.

As elongation continues there will be only a narrow, peripherally placed sheath of cytoplasm adjacent to the walls and in the region around the nucleus. All other parts of the cell are vacuolated. Vacuolation of the antipodal cells is thus one of the causes of a distinct polarity in the cells throughout their existence.

Ultrastructural investigations of the walls of antipodal cells show that they are built up of an electron-translucent matrix with more or less frequent cellulosic fibrils (Fig. 5B, 7A–B); therefore, the chief impression is that the walls have a loose texture, but they are further diversified dependent upon which cells they separate. Three situations are possible because the neighboring cell can be another antipodal cell, the central cell or, later, the developing endosperm, or the neighboring cells of the placental region of the nucellar tissue (Fig. 1).

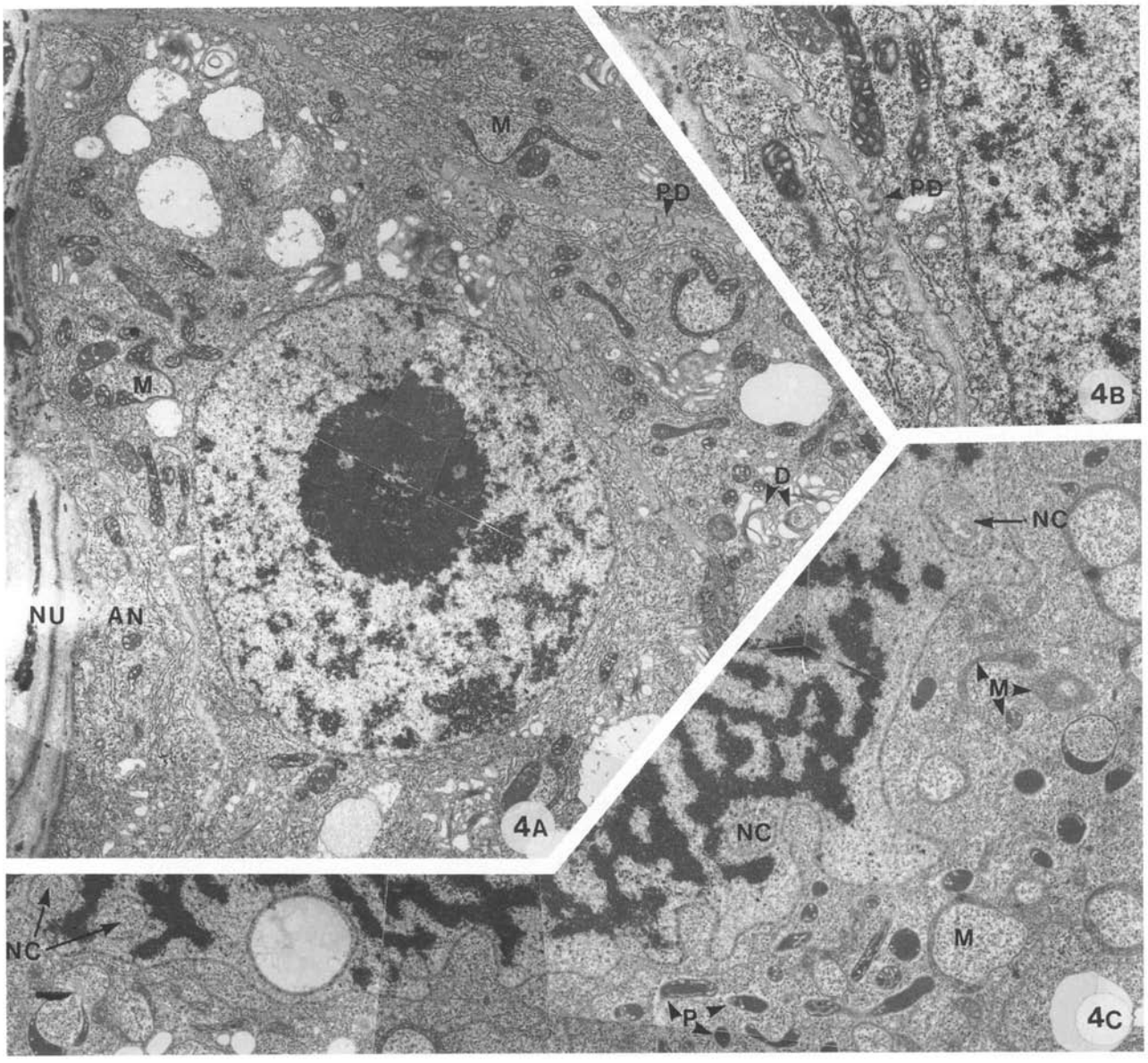


Fig. 4A–C Ultrastructure of antipodal cells. **A** A spherical antipodal nucleus with nearly uniform distribution of heterochromatin in the nucleoplasm (48 h.b.p., as in Fig. 3A). ×7200. **B** Details of one of the walls in Fig. 4A with several plasmodesmata. ×14400. **C** A small portion of an antipodal nucleus as late as 43 h.a.p. (as in Fig. 3E). The nucleus has more compact clusters of heterochromatin, and the circumference of the nucleus is very irregular with small intrusions of the nuclear membrane and even small invaginations with organelles inside. ×7200

The latter situation is further complicated by the fact that this represents the boundary zone between the gametophytic and the sporophytic generations. Even when antipodal cells adjoin each other, wall structures differ depending on their orientation (Fig. 5). Walls parallel to the longitudinal axes of the embryo sac (Fig. 6A) are thinner than walls oriented transversely to the ES and perpendicularly to the placental region as well as the central cell and endosperm (Fig. 5C–E). These perpendicular walls were generally, in the entire period of the investigation, thicker and more uneven in thickness than walls parallel to the axis of the embryo sac. Furthermore, the course of transverse walls (Fig. 5C, 7C) as well as walls parallel to the axis of the embryo sac (Fig. 4A–B) are very sinuous in the time before they elongate.

The transverse walls are thickest in the neighborhood of the nucellus, and from there the cell walls thin gradually as they approach the central cell (Fig. 5E). Only where 3 to 4 antipodal cells come across, are the walls of the corners thick (Fig. 5B,D); sometimes, there will be intercellular spaces in such corners. Plasmodesmata frequently occur in these transverse walls, especially in the middle region (Fig. 4A, 5D).

Changes occur in wall length as well as direction by the elongation of the cells, particularly in the transverse walls, and therefore the walls are less undulated and more homogeneous in thickness. However, later in de-

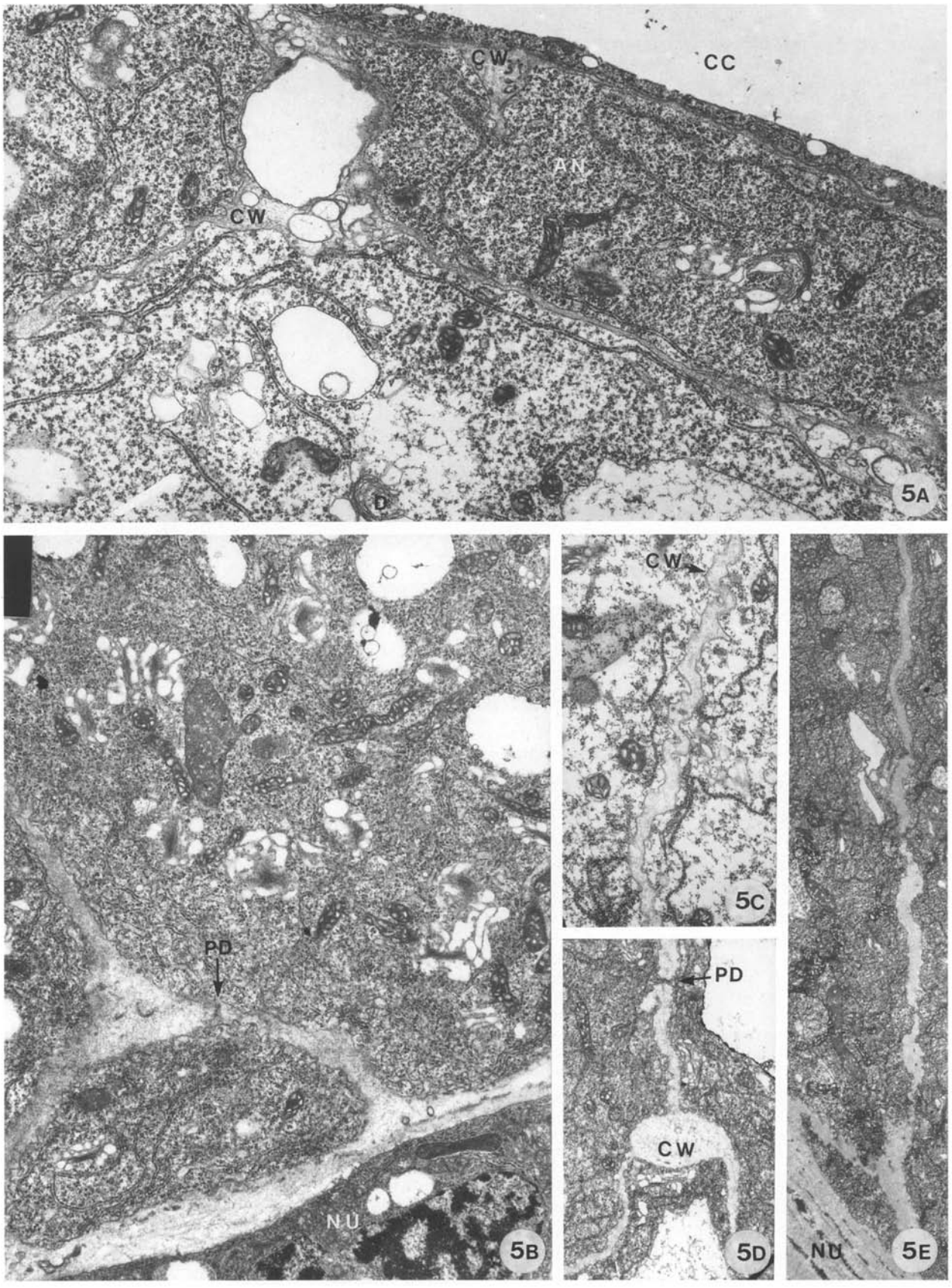


Fig. 5A–E Walls separating antipodal cells. **A** Longitudinal walls between antipodal cells near the central cell. The transverse walls are very uneven in thickness and large vesicles are found inside the walls (48 h.a.p.). × 14400. **B** Three juxtaposed antipodal cells. Uneven, transverse walls with plasmodesmata. (0 h.a.p.). × 9600. **C** A very sinuous wall part of a transverse wall (30 h.a.p.). × 14400. **D** A small part of the transverse walls of antipodal cells (6 h.a.p.). × 7200. **E** A transverse wall, placed perpendicular to the placental region as well as the central cell (20 h.b.p.). × 7200

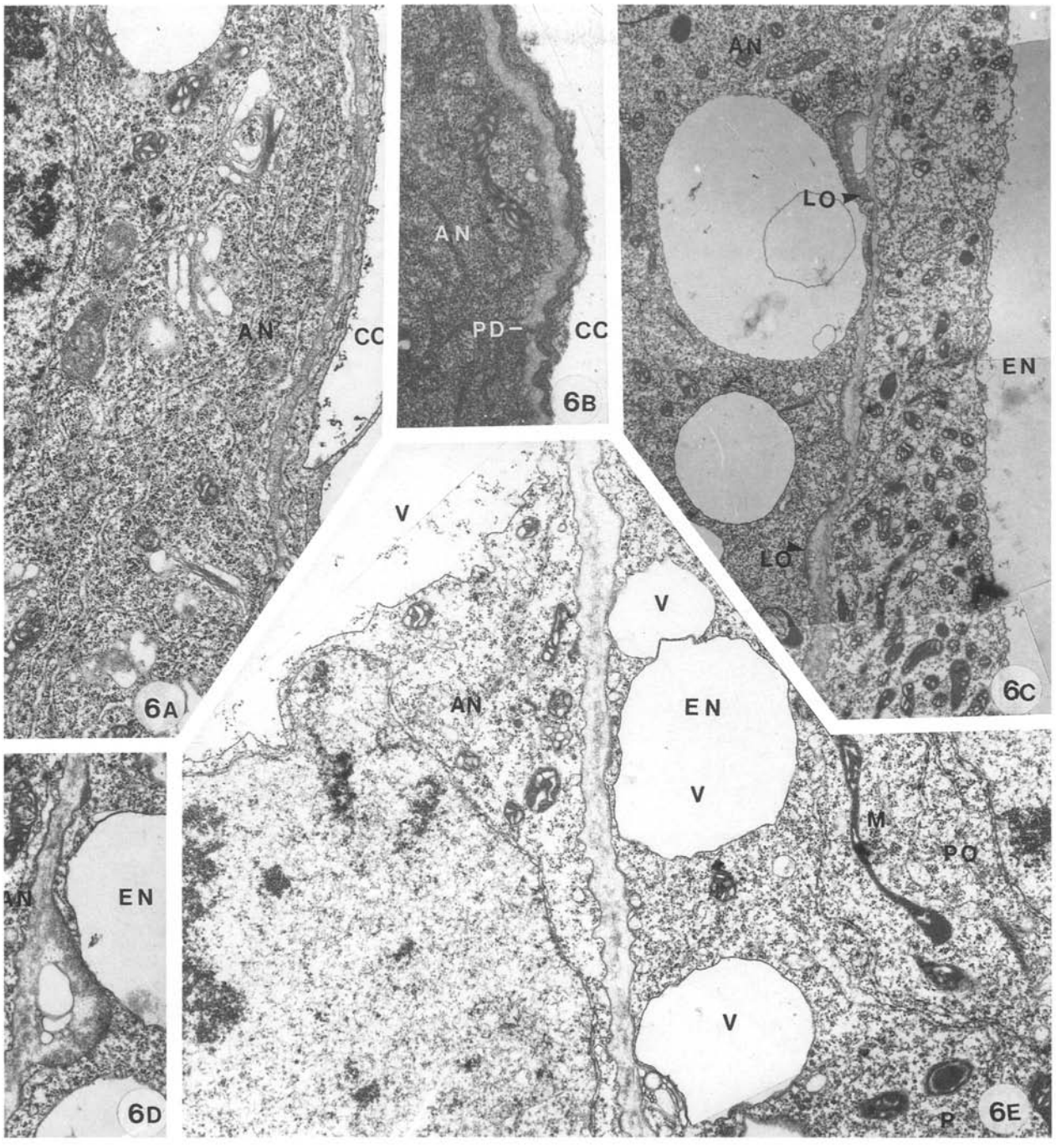
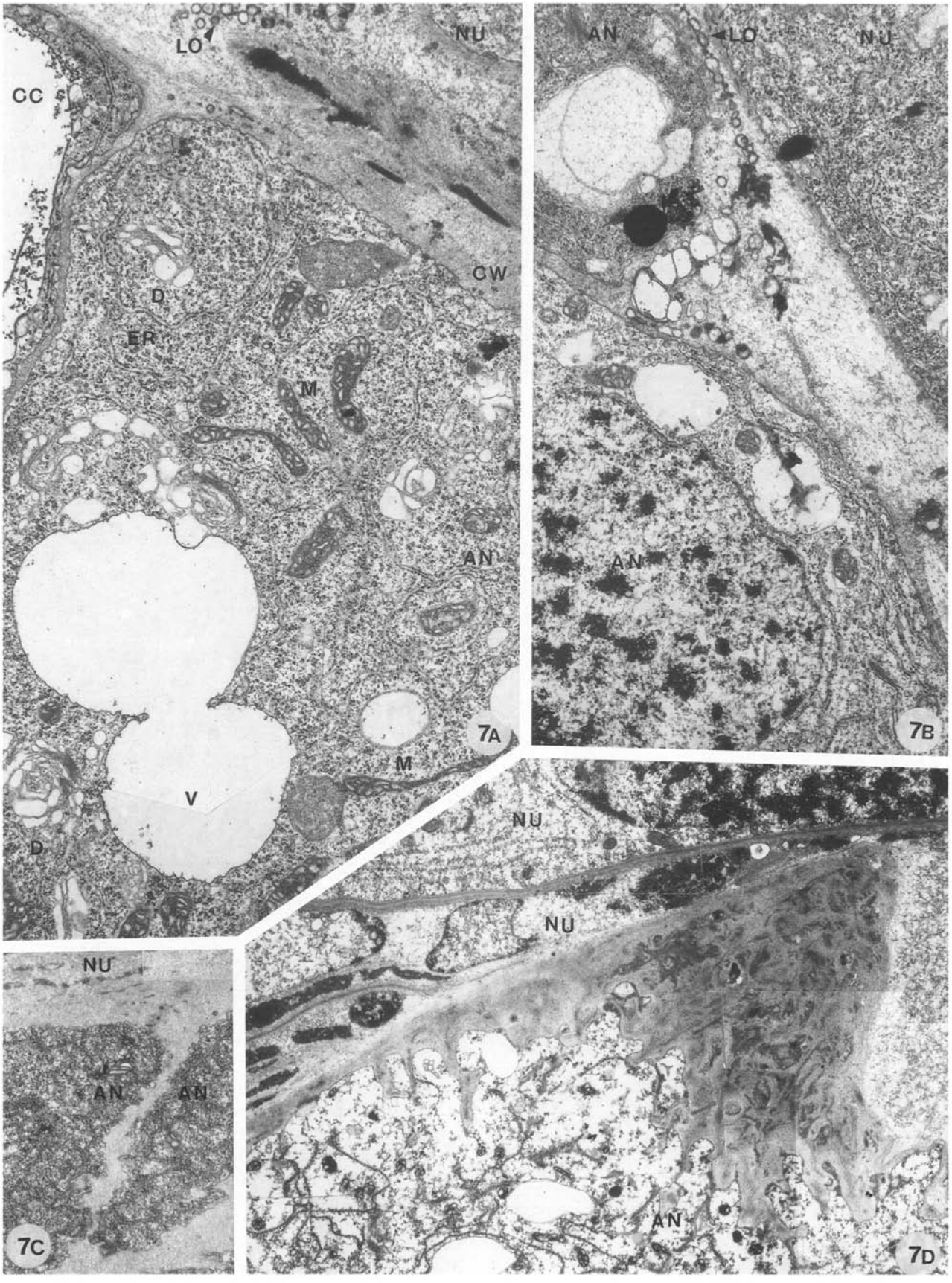


Fig. 6A–C Walls, especially between the antipodal cells. **A** A great part of an antipodal cell. The whole center of the central cell is occupied by a large vacuole, and the cytoplasm is peripheral in a very narrow zone (40 h.b.p.). × 14400. **B** The same situation a *little later* (20 h.b.p.). A single plasmodesma is present. × 19000. **C** Blebs of wall materials with some lomasomes inside an antipodal cell (68 h.a.p.). × 7200. **D** A bleb on the wall from the endosperm side (68 h.a.p). × 14400. **E** A very loose and uneven wall separating an antipodal cell from the endosperm. The antipodal nucleus is near the separating wall and a small portion of an endosperm nucleus is visible. The antipodal cell has one large, central vacuole, whereas the endosperm area has many vacuoles of *different sizes*, dilated cisternae from the endoplasmic reticulum and mitochondria in division (68 h.a.p.). × 14400

velopment (40–50 h.a.p.), wall invaginations are formed now and then and so the walls between juxtaposed antipodal cells still have areas of varied thickness.

The walls in the zone between the antipodals and the central cell are only slightly unequal in thickness. A few plasmodesmata pass through these walls (Fig. 6B).

When the formation of the peripherally placed nuclear endosperm has started, some parts of the bordering wall become thicker and more homogeneous, but still with an apparently loose structure. The occurrence of plasmodesmata is still very rare but some bulges of wall materials are formed and so the plasma membrane en-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larges in these areas and stretches deeper into the cytoplasm from both sides (Fig. 6 C–D).

Inside these bulges or blebs of wall materials there are some lomasomes, small inclusions surrounded by a single membrane, and real vesicles (Fig. 7C–D).

The last neighboring situation is that of the antipodal cells facing the placental region. Here the walls of the antipodal cells are of a loose and open composition but never with plasmodesmata. These antipodal walls are thin originally (48 h.b.p.) but later become thicker and a few wall invaginations will developed deeply into the cytoplasm, particularly where transverse walls join the outer embryo sac wall (Fig. 7D). The amount of wall materials always increases a great deal in such an area (Fig. 8).

From the nucellar side of the boundary, the walls are thick and unequal during the whole period of investigation. From this side more wall materials are added gradually, when some of the nucellar cells degenerate. The cytoplasm of nucellar cells breaks down and remnants of the cells are pressed together just outside the antipodal cells (Fig. 7A,C).

Organelles are numerous in antipodal cells at every stage in the development. Sometimes it is difficult to distinquish mitochondria from plastids. Their shapes are similar but vary from spherical to ovoid, with more variable shapes occasionally seen (Fig. 8, 9); in general, plastids are larger and darker and they have a more electron-dense structure. Furthermore, they appear a little less frequently than mitochondria.

The mitochondria appear in antipodal cells in large quantities from the earliest stages of this investigation (48 h.b.p.). They are distributed throughout the cytoplasm, but the majority are found in the cytoplasm just inside the walls separating the antipodals from the nucellar tissue. Their shapes vary widely from spherical-ovoid to elongated rod-shaped, to annular profiles or cuplike shapes with rims containing cristae and thin cristae-free bases (Fig. 8A–C). These bases are sometimes strongly stretched out with a very thin central part; such dumbbell-shaped mitochondria show that there are frequent divisions of mitochondria. Divisions are a simple parting process after contact is made between parts of the surface of the inner membrane in the long, narrow passage.

Roundish, ovate mitochondria are placed on both sides of the transverse walls separating the adjacent antipodal cells, whereas mitochondria in various shapes are spread out in the cytoplasm in the middle of the cells. The cristae are often baglike, dilated in the middle of the mitochondria. Not until some days after fertilization (48–50 h.a.p.) do the numbers of mitochondria decrease. Degenerations occur in some of them, but, nevertheless, others are still dividing.

Normally, plastids are distinquishable from mitochondria because the inner compartments are more compact and dense. They are often large and ovate to elongated in shape. The thylakoid membranes are sparse and irregularly developed and starch grains are not deposited in the plastids of antipodal cells (Fig. 9).

Plastids are distributed throughout the whole cytoplasm. Their numbers decrease earlier than numbers of mitochondria. At 24 h.a.p. some of the plastids show signs of degeneration, the inner membrane in some of the plastids being constituted only of a single annular thylakoid membrane later on (Fig. 6E). Such degenerating plastids are most frequent in the cytoplasmic area near the nuclear endosperm. At the same site, much later in development (68 h.a.p.) some plastids of peculiar shapes are visible. They develop something like a budding central part, perhaps a real partitioning in many small plastids, or maybe some sort of degeneration process. True divisions of plastids occur in the days before and just after fertilization. The plastids become deeply furrowed in the middle, sometimes a little terminally to that, and around this constriction a ringlike structure, perhaps a circular bundle of fine filaments aligning themselves circumferentially, becomes visible. A binary fission then takes place and later there are two independent plastids (Fig. 9B–C).

Thus the division modes of mitochondria and plastids are different and, in that situation, it is clearly possible to distinquish between the two types of organelles.

The endoplasmic reticulum (ER) is extraordinarily well-developed and remains so nearly the whole period of this investigation. Throughout the cell at 40 h.b.p., there are often long profiles of ER parallel to the walls (Fig. 5A, 7B); nuclei or the vacuoles (Fig. 4A) are distributed in the cytoplasm (Fig. 6A). Often the cisternae show dilations. The ER is of the rough type (RER) over nearly all of the cell and the ribosomes form many polysomes in contact with the membranes (Fig. 9B). About the time for pollination (0 h.a.p.) especially the ER cisternae near the walls against the central cell have dilations at the ends. At that time, there are some annularly arranged cisternae and sometimes there are lipid bodies in the center of the rings. These ring-shaped ER are often of the smooth type (SER). Most of the time, the RER constitutes a regular stack of cisternae along the walls against the central cell, but sometimes they are arranged perpendicularly to the walls. About 24 h.a.p., the course of the RER is a little more irregular through

Fig. 7A–D Antipodal cells facing the placental region. **A** An area between the central cell, an antipodal cell, and nucellar tissue in the placental region. Fibrillar wall material from demolished nucellar cells makes the boundary very thick. The string of lomasomes indicates the limit between the walls of the antipodal cells and the nucellar cells outside the embryo sac (40 h.b.p.). ×14400. **B** Antipodal cells facing an intact nucellar cell. Once again the string of lomasomes indicates the limits between the cell types (40 h.b.p.). ×14400. **C** A thick mass of material from the placental cells is gathered outside the antipodal cell. Between the two antipodal cells, the walls are of very different thickness (20 h.b.p.). ×14000. **D** Wall invaginations with many fingers deeply invaginated into the antipodal cell. The nucellar cell nearest to this antipodal cell has begun degenerating, while the next cell is still intact (50.5 h.a.p). ×7200

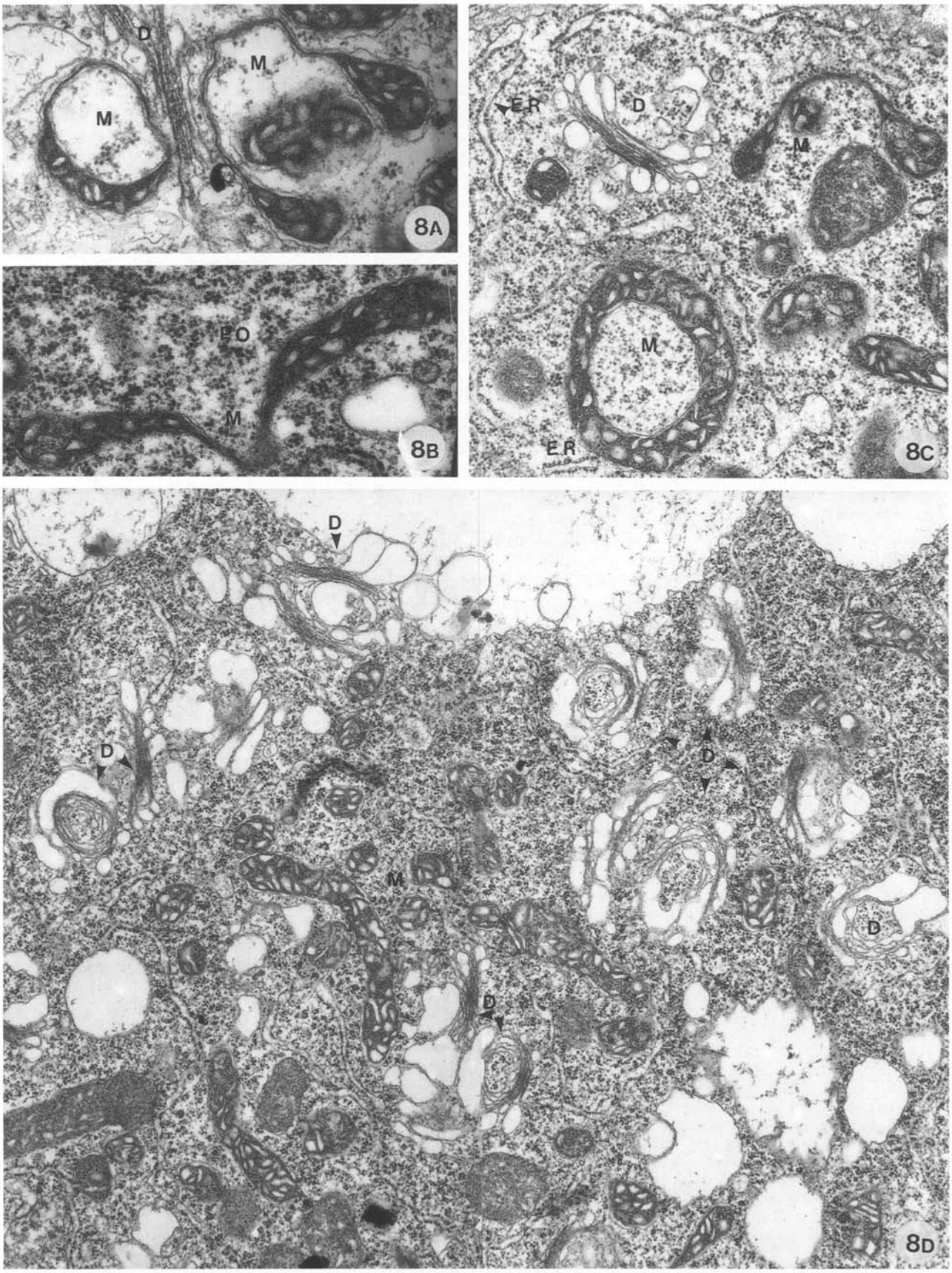

Fig. 8A–D Organelles in the antipodal cells. Note the especially numerous mitochondria and dictyosomes. **A** Mitochondria with thin crista-free bases (40 h.b.p.). ×38000. **B** A dumbbell-shaped mitochondrion in simple division (0 h.a.p.). ×38000. **C** Many different organelles; RER with dilated cisternae (40 h.b.p.). ×28000. **D** Enormous amounts of dictyosomes throughout the cytoplasm (40 h.b.p.). ×20000

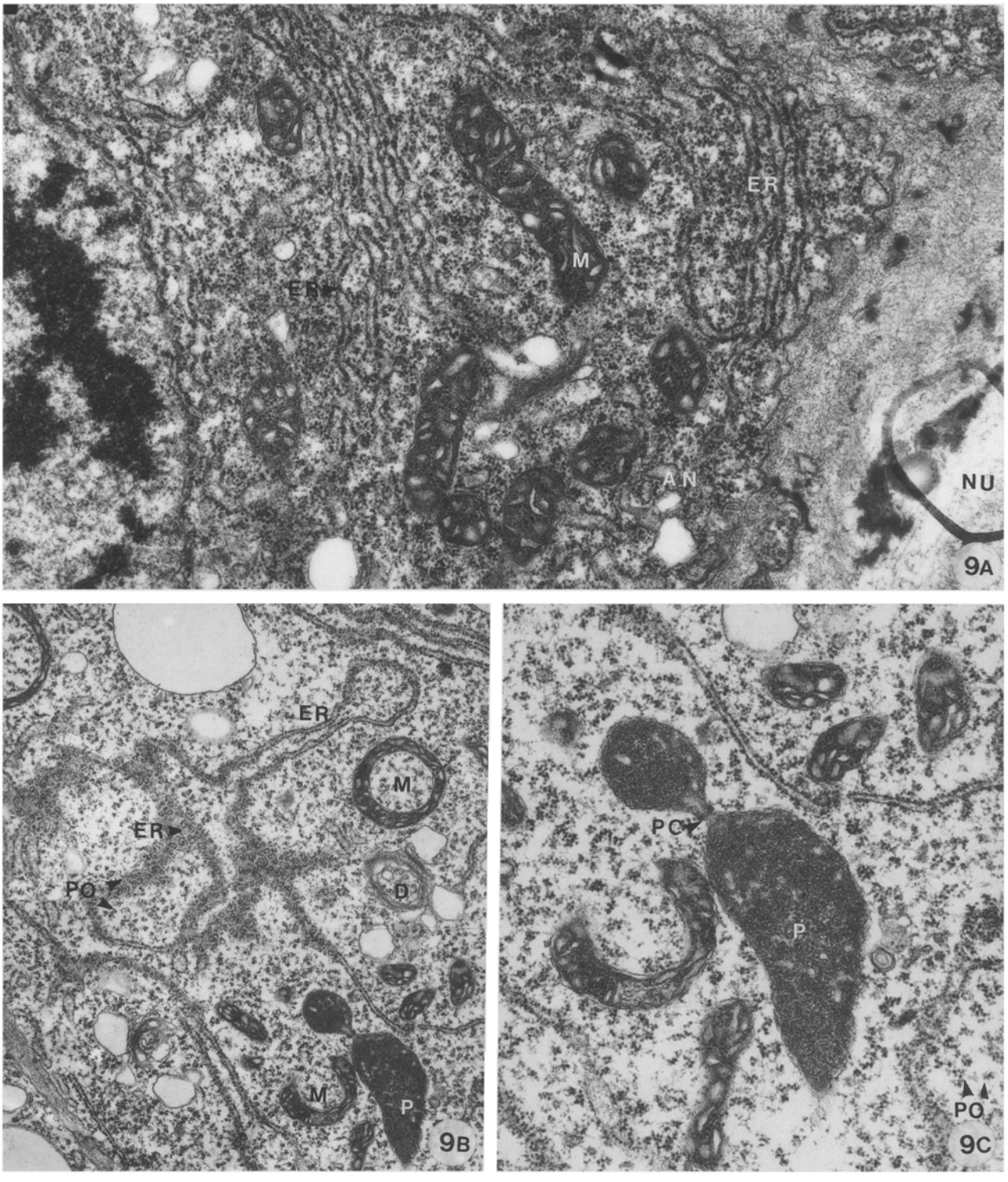

Fig. 9A–C Organelles in antipodal cells, especially plastids and RER. **A** An inner corner of an antipodal cell near the nucellus. The cytoplasm is very dense with abundant RER and polysomes (0 h.a.p.). × 28000. **B** Cytoplasm in the middle of an antipodal cell with a dividing plastid and RER with polysomes on the membranes (48 h.b.p.). × 14000. **C** The same dividing plastid as in **B**. A ringlike structure is visible around the smallest part of the plastid constriction. × 28000

the middle of the cells, but there is still much RER and now and then there are extensions or formations of isolated bags or vesicles. About 68 h.a.p., a diminishing in the amount of RER occurs but there are still lots of cisternae parallel to the walls against the endosperm, with many dilated areas in the middle or in the ends of the cisternae and continuous formations of vesicles from the ends.

The amount of dictyosomes is enormous in the earliest period (Fig. 8D). There are dictyosomes throughout the cytoplasm 48 h.b.p., mostly with 3 to 4 cisternae, crescent-shaped or pitcher-shaped or of spherical appearance with dilated rims and vesicles of different shapes and dimensions. About 40 h.b.p., some polarizing begins and, therefore, especially in the cytoplasm just inside the nucellus cells and in an opposite direction to the central cell, the amount of dictyosomes is higher than in the middle of the antipodals where the number is more moderate.

About 20 h.b.p., some flattening of dictyosomes occurs and the cisternae occur as a pile of pancakes with a weak dilation of the rim and a moderate production of vesicles; then, the production increases in the next hours and at the time for pollination (0 h.a.p.) there are many vesicles near the walls of the nucellar region. The dictyosomes themselves grow to 5–6 cisternae in the stack and the dilation of the rims will increase as does the formation of vesicles. Therefore, during the whole first day after pollination and fertilization, rows of vesicles are standing perpendicular to the walls facing the nucellar cells and against the walls separating the antipodal cells in this region too. The rims of the cisternae are strongly dilated before the formation of vesicles (Fig. 8B).

In the zone around the nucleus the frequency of dictyosomes with vesicles also increases. The amount of dictyosomes later decreases slowly as does the formation of vesicles; but even in the latest part of this investigation (68 h.a.p.), there are still many dictyosomes; the production of vesicles still occurs but with less frequency.

The amount of ribosomes increases steadily in the whole period of investigation (48 h.b.p. to 68 h.a.p.). In earliest stages, there is electron-dense ground plasm with many ribosomes free of each other (Fig. 6A), but there soon become more and more helical polysomes and they are found all over the cytoplasm as well as in connection with all the ER (Fig. 9B–C). This condition is maintained, with many ribosomes, mainly as polysomes, occuring in the cells.

Discussion

The functions of the antipodal cells have been discussed in several reports. No one doubts that, if the antipodal cells persist, they perform an important role in the supply of nutrition to the whole megagametophyte and the embryo in particular, so they are indirectly involved in the formation of the embryo. The ultrastructural appearance clearly confirms the opinion of a function in nutrition because the cells possess substantial amounts of dense cytoplasm with numerous organelles of every kind throughout their life span. These relations are common for *Hordeum* (The present work; Cass and Jensen 1970; Cass and Peteya 1986) and the few other genera which have been examined with TEM: *Stipa* (Maze et Lin 1975), *Zea* (Diboll 1966, 1968; Lammeren 1987), and *Triticum* (You and Jensen 1985). Both for *Stipa* and *Zea*, it is emphasized that the antipodal cells are the most organelle-filled cells of the embryo sac; for *Hordeum*, it seems they are even more filled than the synergids (Engell 1988).

In all genera except *Zea*, the antipodal tissue is composed of several uninucleated and long persistent cells. In *Zea*, the antipodal cells become 1–4-nucleate and stay constantly in the originally chalazal position. The antipodals in the other genera change from a chalazal to a lateral position associated with longitudinal stretching of the central part of the megagametophyte in the chalazal direction. Because of this growth, the position of the antipodal tissue will become lateral and near to the placental region, a region rich in vascular supply and such a change seems to be an improved position for effective transportation of nutrients to the embryo sac by the antipodal cells.

In *Hordeum*, the original three cells increase in size and number (a result of divisions), before fertilization of the egg and the central cell; and after fertilization, there is a remarkable increase in size (not numbers). This growth of both the nucleus and nucleolus and the individual cell occurs in spite of the fact that the antipodal cells are not of biparental origin but carry only the haploid complement of female chromosomes. Brink and Cooper (1944) earlier showed by crossings of *Hordeum jubatum* × *Secale cereale* that the behaviour of antipodal cells was quite different because, in such cases, they do not change very much in size but their numbers increase after fertilization and their functions are shown to be different also.

The ultrastructure of antipodal cells in barley as well as in the other investigated species indicates that they are cells with high metabolic activity and the structure is indicative of a high rate of material transport. Characters that point to this conclusion are both the proliferation of cell numbers up to several antipodals per ovule, as well as individual cells with substantial amounts of dense cytoplasm from the earliest stages, with numerous mitochondria, ribosomes, polysomes, rough endoplasmic reticulum, and the formation of wall invaginations. From the present investigation, it seems clear that even in later stages the amount of organelles is still enormous, because both mitochondria and plastids are dividing, and first, when the endosperm becomes cellular, the antipodal cells quickly decline.

For both *Triticum* (Bhatnagar and Chandra 1975) and *Zea* (Diboll 1966, 1968) it is reported that antipodal cells are functioning until about the cellularization of the endosperm, similar to the situation in barley. In *Stipa* (Maze and Lin 1975), the degeneration may be a little earlier. The papillate cell walls that are common for them all may be related to a suspected synthetic activity and/or to a solute flux of large amounts of materials from or into the adjacent tissue. One of the first functions of this multicellular antipodal tissue may be production and secretion of enzymes for the degradation of the nucellar cells just beyond the embryo sac wall.

The arrangement of the great amount of dictyosomes with numerous vesicles along the periphery in the earliest period and about the time of fertilization (except in *Stipa* [Maze and Lin 1975] where the dictyosomes have only few visicles), shows that the frequency of these organelles is greatest where the antipodal cells are adjacent to cells of another type, e.g., against the nucellar cells and against the central cell or, later, the coenocytic endosperm. Thus the dictyosomes are arranged in a bipolar position.

This bipolar position and the very active production of vesicles sometimes in rows perpendicular to nucellar tissue and combined with many ER cisternae indicate that the antipodal cells are involved in certain secretory activities; and furthermore, the abundance of mitochondria in the same areas confirms a high supply of ATP for this presumed activity. The cells are able to secrete enzymes into the adjacent nucellar cells, and such a process seems to happen because the breakdown of the nucellus to a mass of degenerate cells looks more like an enzymatic demolishing than merely a mechanical destruction by pressure from the growing gametophyte (especially the antipodal cells). Some cells are totally degenerate; still others look functional for a long period in spite of the fact that adjacent cells are degenerating. The nucellar lysate may then be absorbed into the antipodal cells and later delivered to other cells of the embryo sac.

The development of wall invaginations, together with extensions of the plasma membrane, are important formations because they facilitate absorption. Plasmodesmata have never been observed in the walls separating antipodal cells from nucellar cells in barley (Engell 1991b) or in other grasses, but such connections are postulated between the chalazal part of antipodal cells and the nucellar tissue outside the gametophyte in *Gasteria* (Willemse and Kapil 1981), *Capsella* (Schulz and Jensen 1972), *Jasione* (Berger and Erdelska 1973), *Helianthus* (Newcomb 1972), and *Spinacia* (Wilms 1981). The antipodal cells may function as the metabolic center for the absorption and elaboration of nutritive materials to the embryo sac and this function appears to continue from the maturation of the gametophyte until cellularization of the endosperm is started.

Inside the cells there are large quantities of ribosomes free or gathered in polysomes and, especially in barley, the polysomes are very numerous. Ribosomes and polysomes are often in contact with the ER and the occurrence of SER is sparse except in the early stages where the formation of ER especially occurs. Circular sheets of ER are observed near the end of the cells adjacent to the central cell in *Stipa*, *Triticum* and *Hordeum* and, in the middle of some of the wheels in barley, lipid bodies are sometimes found. The abundance of ribosomes and ER in the cells indicates protein synthesis, and the distribution of mitochondria is interpreted as evidence of a high rate of respiration.

The plastids (very often in irregular shapes in all investigated grasses) are distributed throughout the cells, but their degeneration starts in the region near the coenocytic endosperm, perhaps indicating a function as potential polysaccharide-producing structures important for the growth of future walls in the endosperm.

The materials absorbed to and elaborated in the antipodal cells will then be transported to the developing endosperm. Some of the material will be transported as symplastic transport through the few existing plasmodesmata. This is a common phenomenon in the investigated species, but most af the nutritional material is forced to go through the walls. This transport is facilitated by the loose structure of the walls and the invaginations developed from both sides of this pathway. Perhaps all the flow is metabolites from nucellus, through the antipodal cells to the endosperm, maybe there is furthermore a special flow of growth-controlling hormones, synthesized in the antipodal cells themselves, to the endosperm. The investigations of Brink and Cooper (1944) indicate that the antipodal cells are involved in control of the earliest endosperm initiation; such a control function can stop the formation of endosperm in some hybrid seeds and then, with little or no formation of endosperm, the whole seed will soon stop its growth and the hybridization is more or less unsuccessful.

The conclusion is that ultrastructural investigations of the antipodal cells in grasses suggest that there are several steps in the area of function of these cells: (1) they are able to synthesize enzymes for the breaking down of nucellar cells; (2) they are able to absorb components from that degradation and, further, to absorb material transported to the ovule by the vascular supply; (3) they are producing nutrients; and (4) they are able to transport metabolites to the endosperm. Most of these functions end at the time when cellularization of the endosperm starts and the antipodal cells disappear.

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