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Callose and osmiophilic material deposition into walls of developing megagametophyte in *Stellaria*

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Abstract

Extensive rough ER cisternae are developing and disappearing during each of intermitotic periods in two and four nucleate embryo sac and synergids. The synergid cisternae are transformed into vesicles. Some long cisternae come in close contact with the embryo sac and the synergid walls. Numerous osmiophilic droplets from the cytoplasm seem to be incorporated into the walls during the period of wall-cisternae relation.

INTRODUCTION

In many plants with a *Polygonum* type of embryo sac development, the cell walls of meiotic cells are impregnated or lined with material containing callose (Rodkiewicz 1970), which disappears after the beginning of the functional megaspore growth. In nucleate stages of the embryo sac in *Stellaria* a new component seems to be built into the cell wall. There occur a large number of osmiophilic droplets which appear to be incorporated into the wall. Later similar droplets are visible in a particular stage of synergid wall development (Kudlicka et al. 1981).

MATERIAL AND METHODS

Ovules of *Stellaria media* (*Caryophyllaceae*) grown in a grrenhouse were fixed for 24 hours in 3% glutaraldehyde at pH 6.8 in cacodylate buffer and postfixed in 2% OsO_4 for 12 hours at 4°C. Following dehydration, ovules were embedded in Epon. Ultrathin sections were countrestained with uranyl acetate and lead citrate and examined in a Tesla BS 613 electron microscope.

RESULTS

The embryo sac of *Stellaria media* is of the monosporic, *Polygonum* development type (Figs. 1-9, 30). In an ovule a subepidermal archesporial cell differentiates giving rise to a parietal cell which subsequently is divided by a perpendicular wall (Flg. 1) and a meiocyte undergoing meiotic division leading to formation of a triad or in rare instances a tetrad (Fig. 16). The functional megaspore occupies the chalazal position.

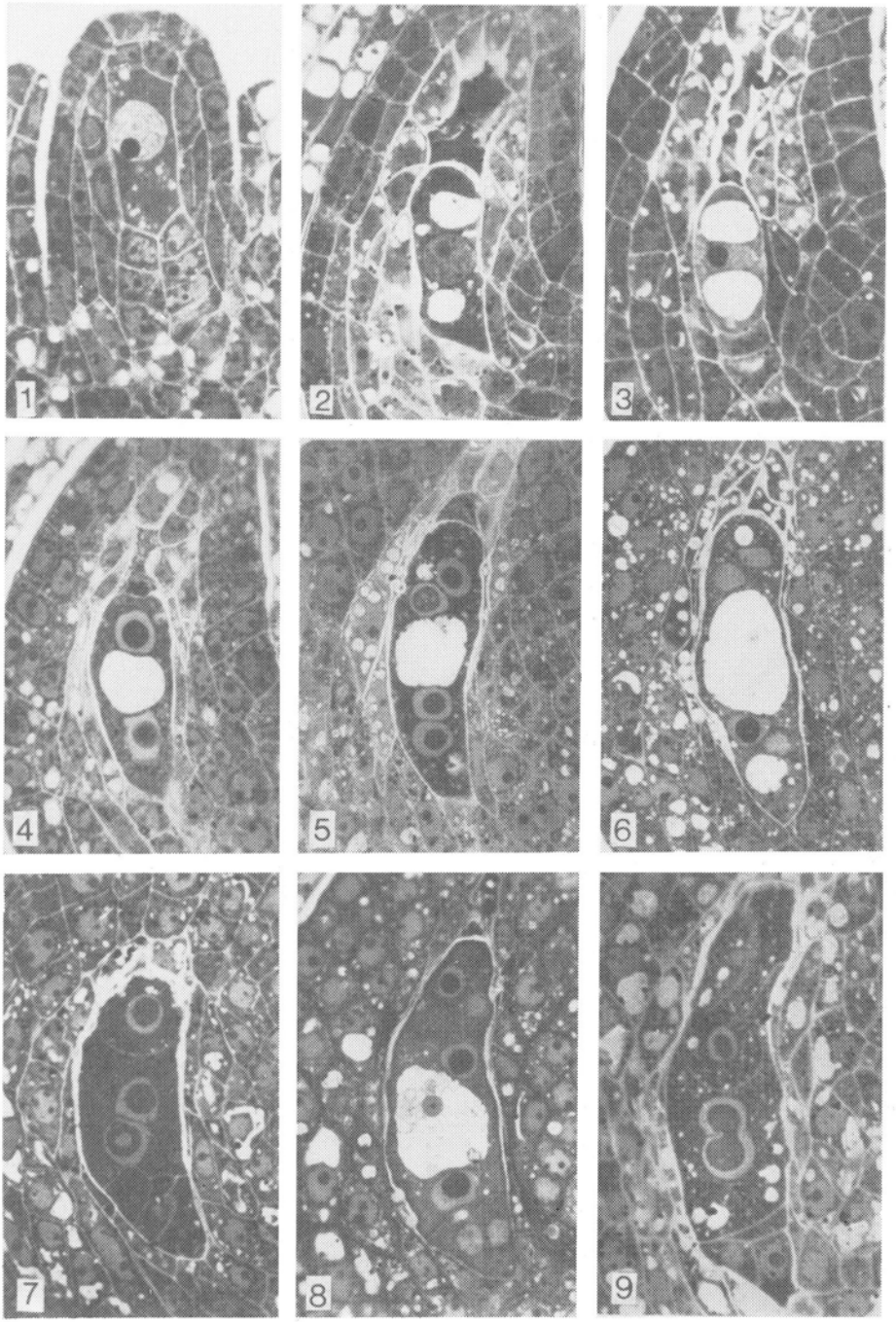
THE MEIOCYTE

The meiocyte is filled by dense cytoplasm with an abundance of plastids and mitochondria, but, during the stages of the first meiotic prophase we examined, with very few dictyosomes and ER cisternae. In the late first prophase there are numerous small vacuoles in both tops of the elongated cell (Fig. 10). Vacuoles contain remnants of denser material and in some there are homogeneous osmiophilic droplets present also in the cytoplasm (Fig. 11).

During the meiotic prophase the meiocyte wall begins to show callose fluorescence after fluorochroming with aniline blue. An especially thick layer of fluorescing material is deposited in the form of a submedian belt (Fig. 12) under the nucleus occupying the upper-central part of the cell (Fig. 10). This belt persists throughout meiosis (Figs. 12-14) and eventually together with a crosswall constitutes the upper portion of the functional megaspore in a triad or tetrad (Fig. 16). The fluorescing material is electrontransparent in contrast with the middle lemella. It seems to be laid by the cytoplasm of the meiotic cell on the extremely thin primary cell wall. The chalazal wall of the functional megaspore is partially devoid of a fluorescing layer and is transversed by plasmodesmata (Fig. 17) which seem to be lacking in the other walls of the megaspore.

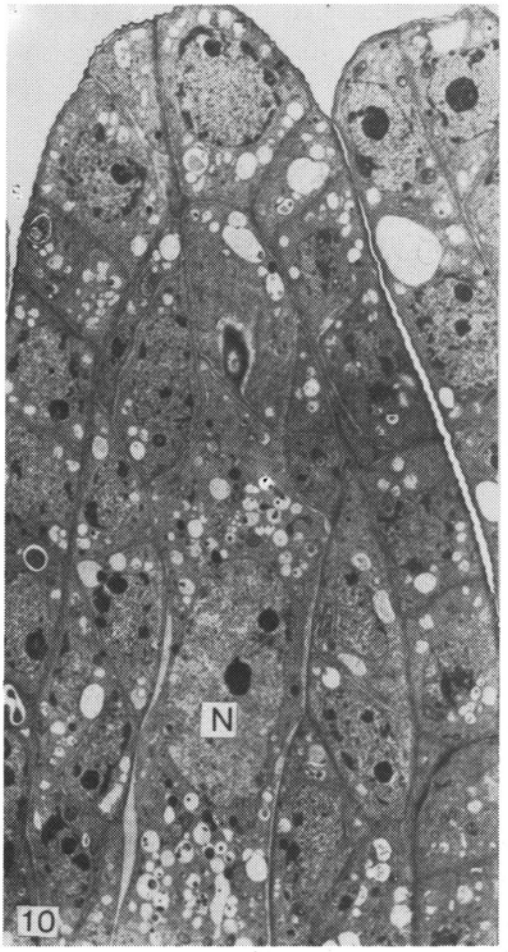
NUCLEATE EMBRYO SAC

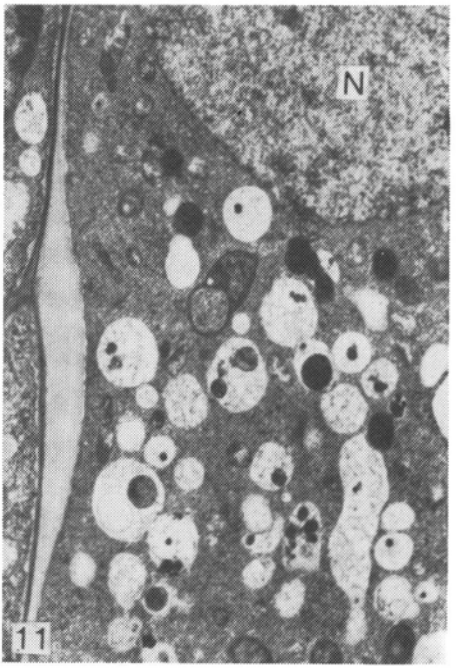
Following meiosis the chalazal/functional megaspore grows, vacuolates and gives rise to a nucleate embryo sac. The development of the eight nucleate stage from the functional megaspore consists of three mitotic cycles and intermitotic phases during which the nucleate embryo sac and its central vacuole undergo appreciable growth and periodical reshapement of some cytoplasm constituents. There occur, in the course of each intermitotic phase, two different types of morphological arrangements in both the cytoplasm and the cell wall. In some preparations the cytoplasm contains numerous cisternae of rough ER which are either mostly long (Fig. 18) or fragmented into pieces of varying length. These shorter cisternae can be found adjacent to the embryo sac wall (Figs. 21-23). In some

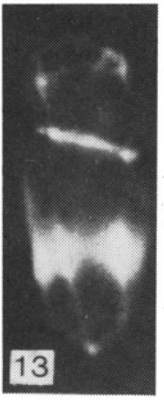


Figs. 1-9. Semi-thin preparations of *Stellaria media* ovules. x 800

Fig. 1. Meiocyte at the first meiotic prophase. Figs. 2 and 3. Embryo sac mother cell and degenerating megaspores. Fig. 4. Two nucleate embryo sac. Fig. 5. Four nucleate embryo sac. Fig. 6. Four nucleate embryo sac, a later stage. In three nuclei, nucleoli are not visible. Fig. 7. Embryo sac, an early cellular stage. Fig. 8. Embryo sac, vacuolate central cell. Fig. 9. Fusion of polar nuclei. Egg cell not vacuolized yet







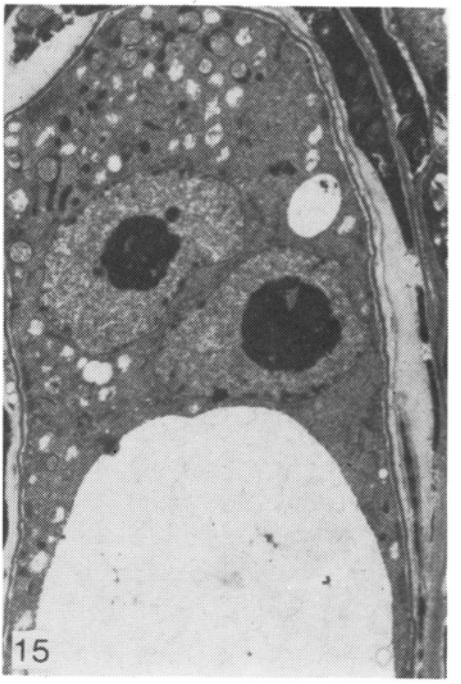
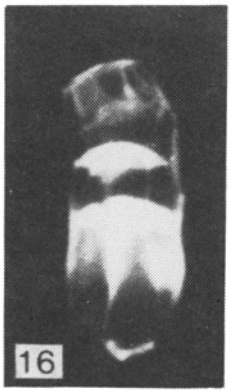


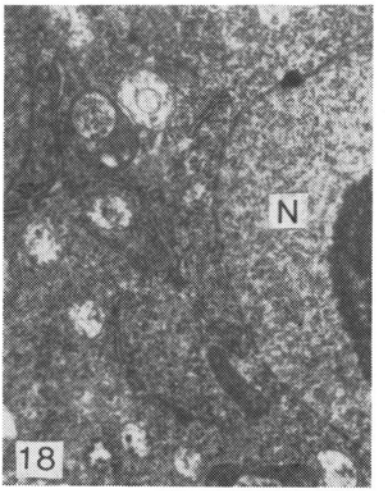
Fig. 10. Ovule with a meiocyte in the first prophase; N—nucleus. x 2300

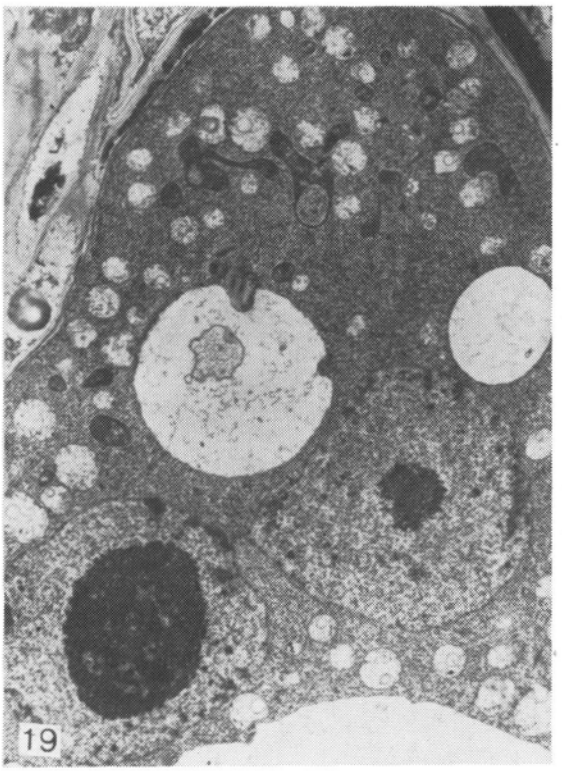
Fig. 11. Subnuclear part of the meiocyte from Fig. 10; thick callose wall, vacuolated cytoplasm, N—nucleus. x 9000

Figs. 12-14. Aniline blue fluorescence of the walls in a meiocyte, diad and triad. x 1600

Fig. 15. Micropylar part of four nucleate embryo sac, plastids (dark) irregularly shaped; circular, translucent mitochondria, no apparent ER. x 7000







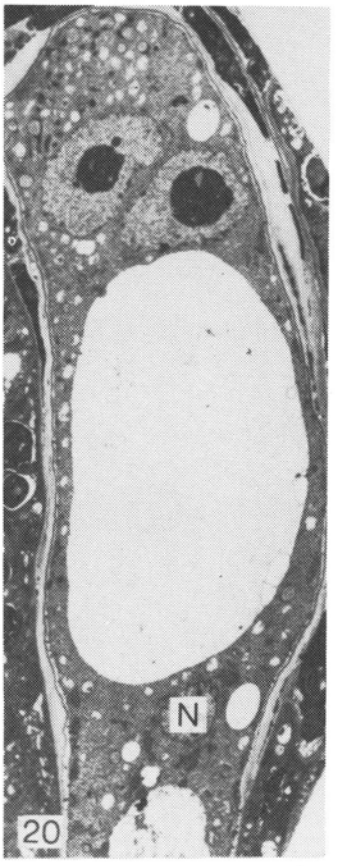


Fig. 16. Aniline blue fluorescence of tetrad callose walls. x 1600

Fig. 17. Chalazal part of the embryo sac mother cell (Me) with plasmodesmata in the wall (arrow). x 6000

Fig. 18. Two nucleate embryo sac with long ER cisternae; N—nucleus. x 6000

Fig. 19. Micropylar part of four nucleate embryo sac, deformed plastids and translucent mitochondria. x 10 000

Fig. 20. Four nucleate embryo sac, only part of chalazal nucleus (N) is visible. x 5000

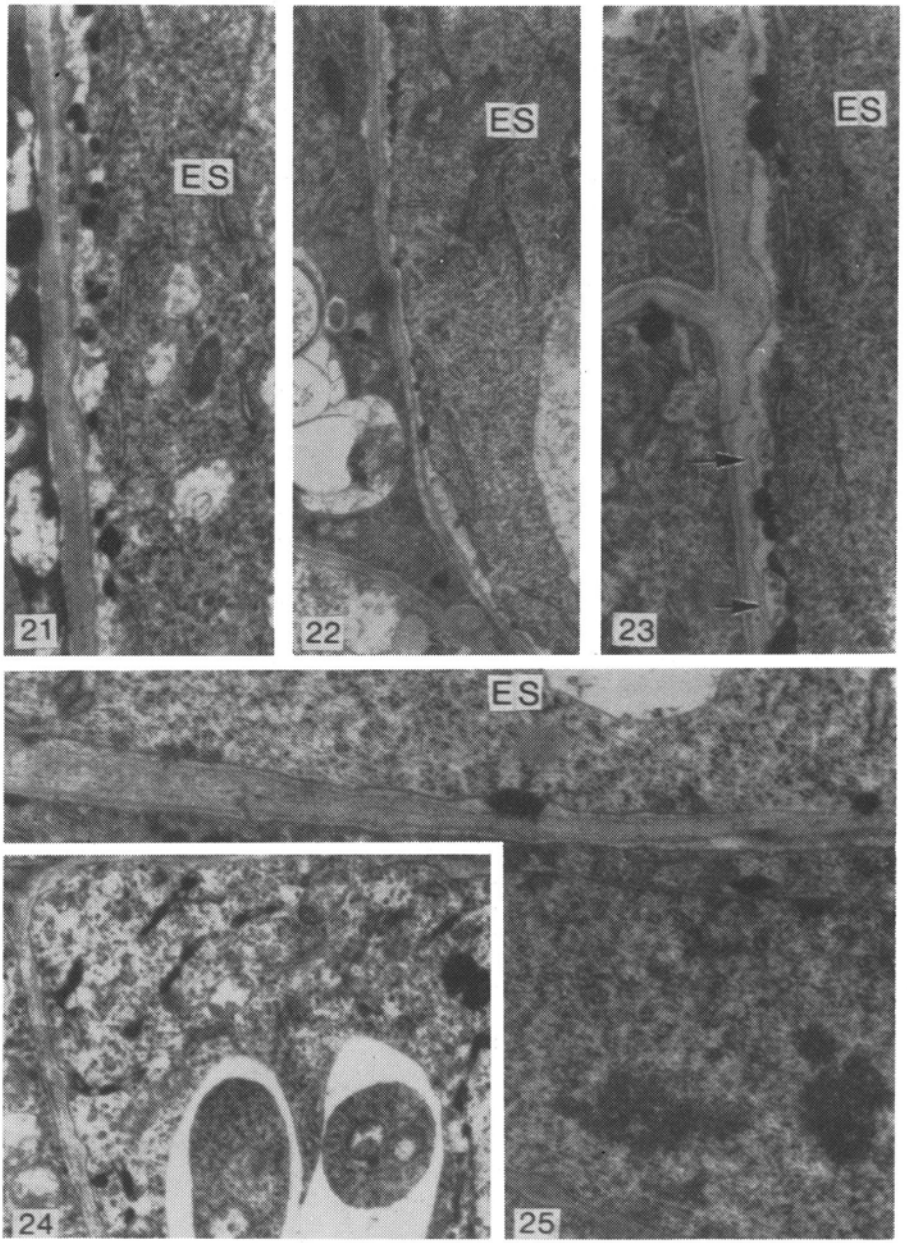


Fig. 21. Two nucleate embryo sac (ES), some short ER cisternae near the cell wall, along the wall a translucent layer, osmiophilic droplets apparent within and outside the layer. x 10 000

Fig 22. Four nucleate embryo sac (ES), some short ER cisternae bordering the translucent layer containing osmiophilic droplets x 8000

Fig. 23. Four nucleate embryo sac (ES), osmiophilic droplets within certain ER cesternae, translucent layer contains osmiophilic droplets and vesicles (arrows) of little internal density. x 15 000

Fig. 24. Nucellar cell bordering two nucleate embryo sac, osmiophilic droplets inside ER cisternae. x 10 000

Fig. 25. Two nucleate embryo sac (ES), translucent layer very narrow, osmiophilic droplets less numerous than in Fig. 21, one of two droplets internal the other external to cytoplasm membrane. x 10 000

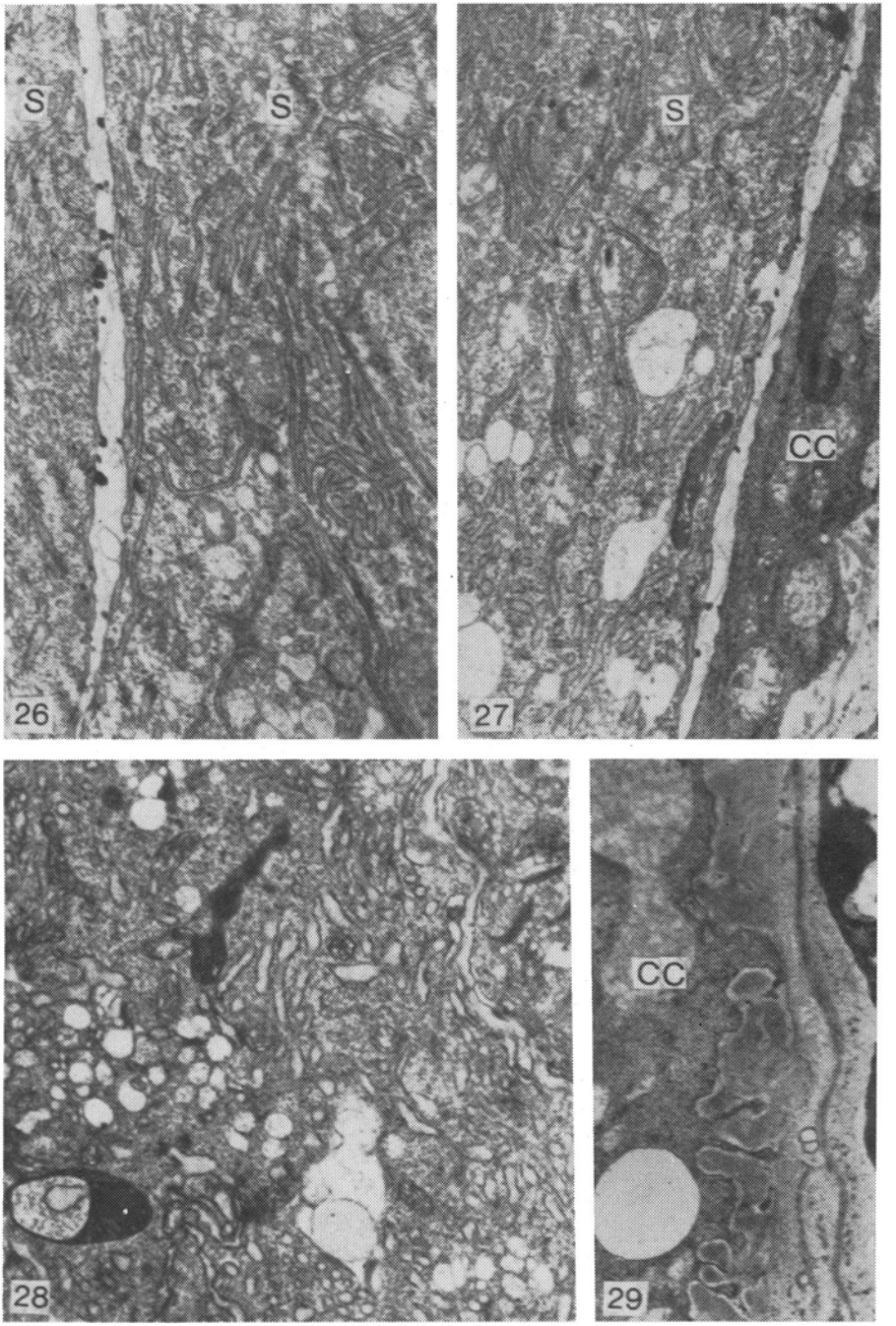


Fig. 26. Differentiating synergids (S), long, rough ER cisternae, some cisternae lie closely pressed to the wall, osmiophilic droplets indented in the wall. x 15 000

Fig. 27. Differentiating synergid (S) and central cell (CC). Some long ER cisternae pressed to the wall. In central cell ER not as apparent as in synergid, osmiophilic droplets in the cell wall. x 12 000

Fig. 28. Vesicular ER in the later stage of synergid development, plastid in the shape of a cup body. x 15 000

Fig. 29. Ingrowths from the central cell wall at the level of egg apparatus in the maturing embryo sac; CC—central cell. x 15 000

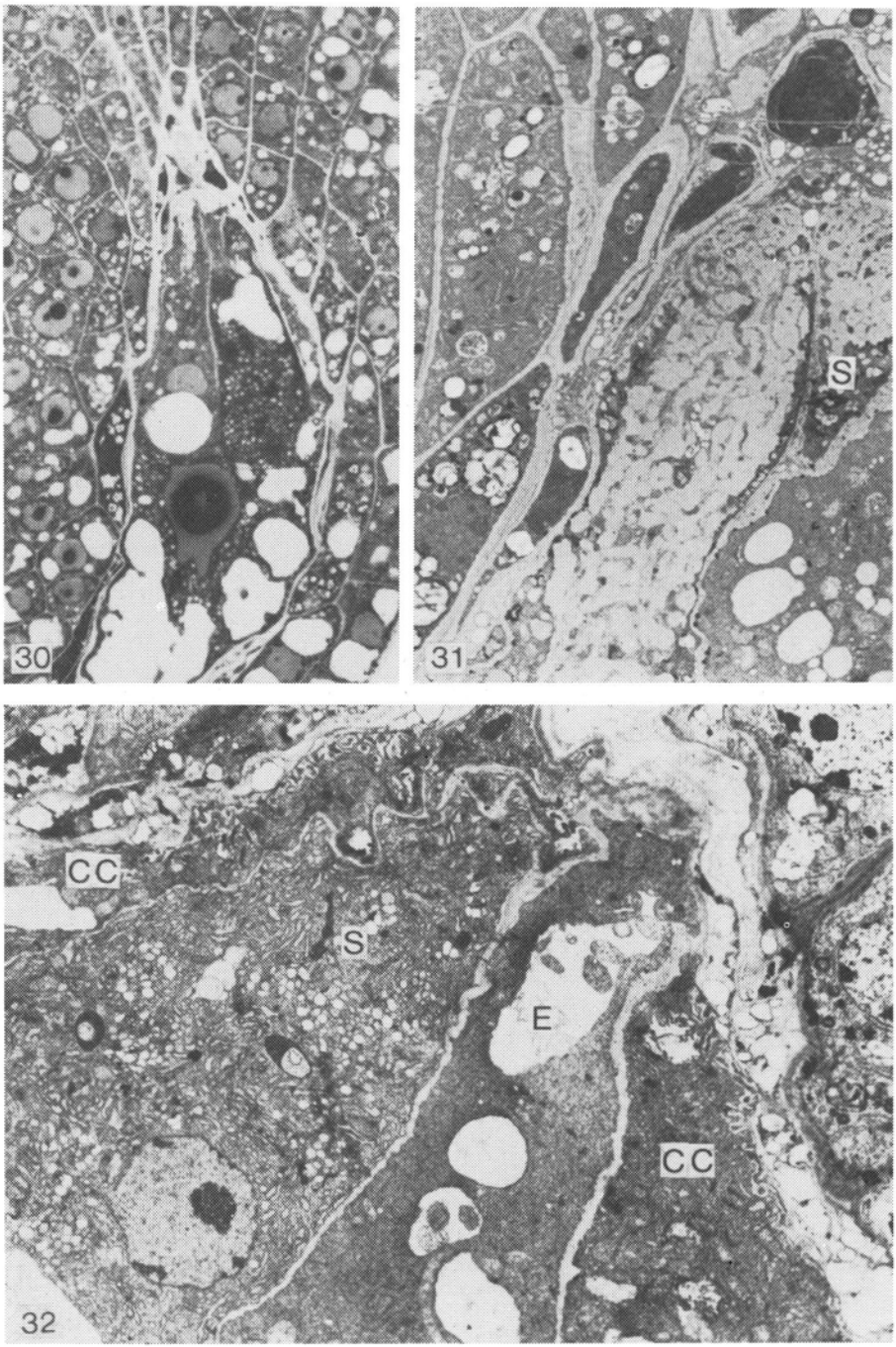
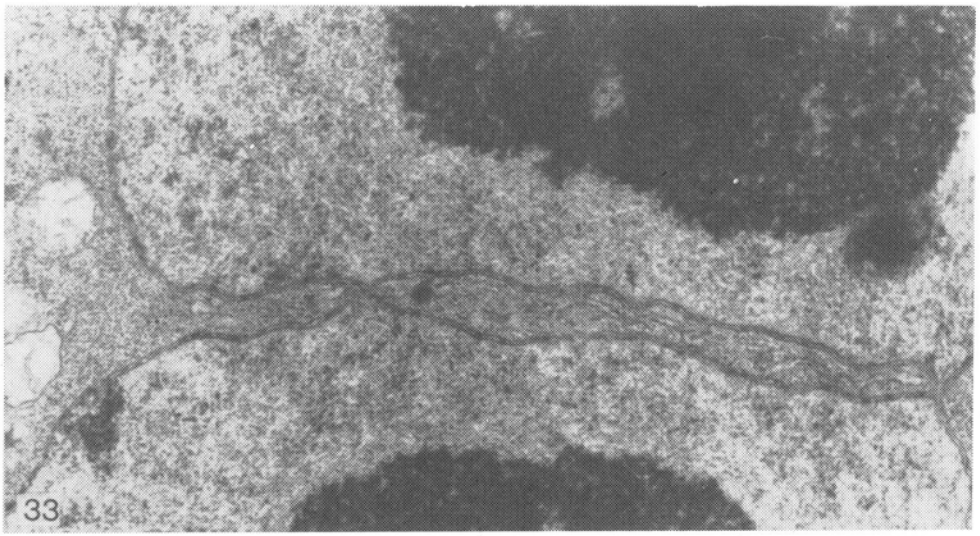
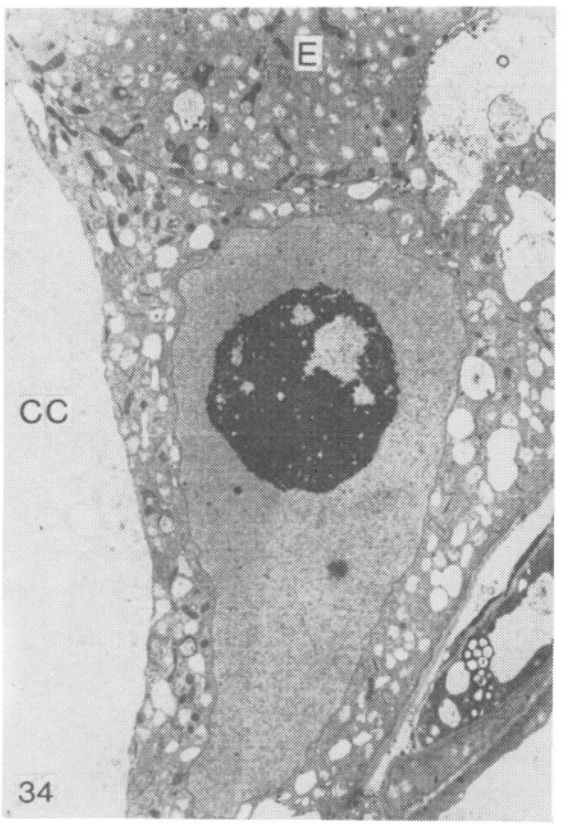


Fig. 30. Semi-thin section of a mature embryo sac. x 800

Fig. 31. Filiform apparatus of the other synergid apparatus (S) only a small part is visible. x 4000

Fig. 32. Micropylar part of maturing embryo sac, a slantwise section does not include the filiform apparatus; S—synergid, E—egg cell, CC—central cell. x 3000





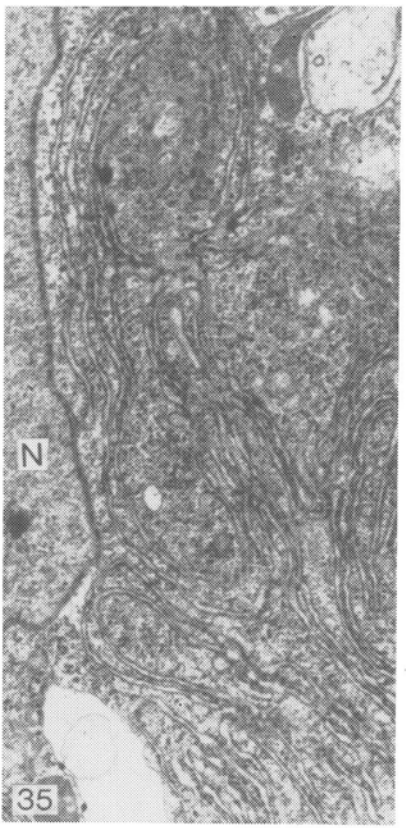
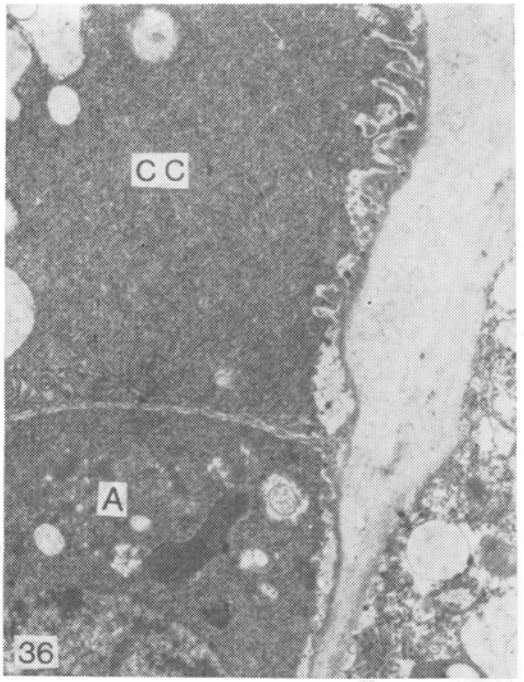
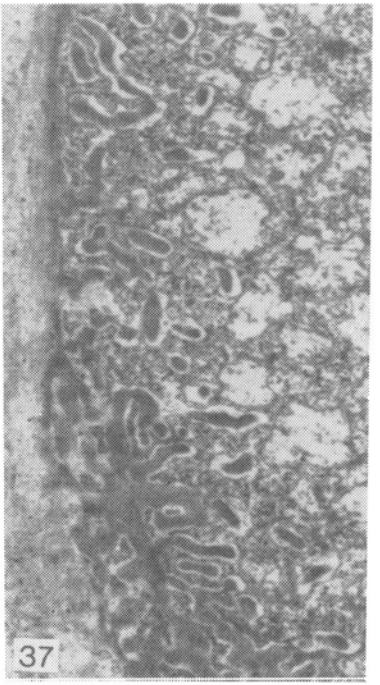


Fig. 33. Fusion of the polar nuclei. x 12 000

Fig. 34. Mature embryo sac with highly vacuolated central cell (CC), part of the egg (E). x 4000

Fig. 35. Whorls of ER in the central cell with two polar nuclei (N). x 20 000





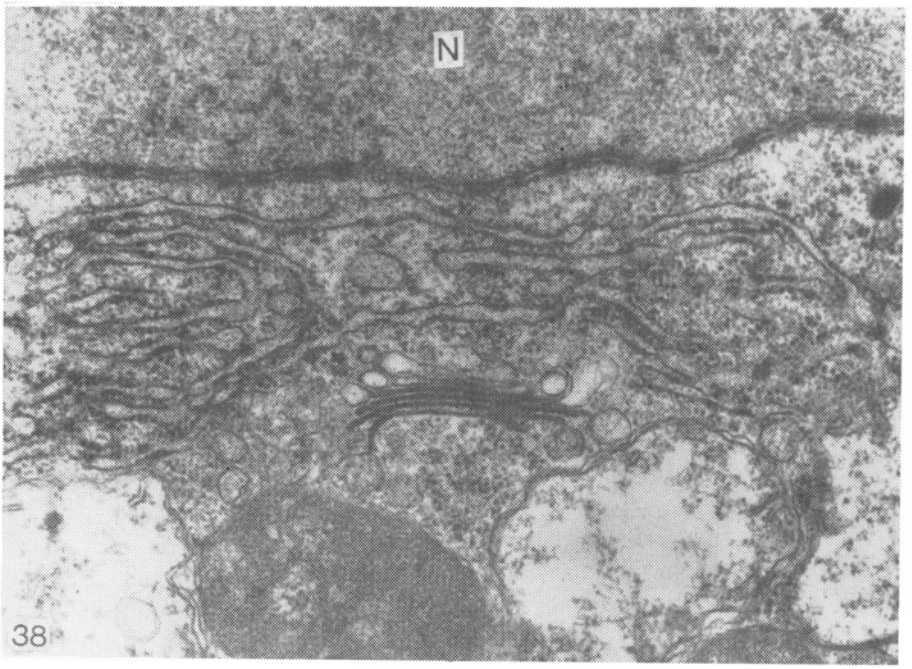


Fig. 36. Central cell (CC) and antipodal (A) in an early cellular stage, corresponding to Fig. 7, the central cell wall with ingrowths. x 5000

Fig. 37. Micropylar part of the zygote wall with labyrinth ingrowths x 8000

Fig. 38. Central cell in a stage corresponding to that shown in Fig. 8, stacks of rough ER cisternae, active dictyosome; N—nucleus. x 25 000

cisternae there are small osmiophilic droplets, but they may be associated with other organelles or exist free in the cytoplasm. Similar droplets may be encountered in ER cisternae of nucellar cells bordering the embryo sac (Fig. 24).

At the stage when ER cisternae are present in the two or four nucleate embryo sac, a number of osmiophilic droplets adhere to the inside and outside of the cell membrane (Fig. 25). These droplets, which occur external to the cell membrane, occupy an electron lucent space stretching along the more dense cell wall layer. Besides the osmiophilic droplets the electron lucent area contains also vesicles exhibiting little electron density (Fig. 23—arrows). In some preparations of the embryo sac there are few short segments of ER cisternae and almost no osmiophilic droplets at the cell wall. This stage seems to be intermediate to the one when the cytoplasm of the nucleate embryo sac is devoid of ER cisternae and an appreciable number of osmiophilic droplets (Figs. 15, 19, 20).

THE CELLULAR EMBRYO SAC

The third round of nuclear divisions is followed by cell wall formation and the cellular stage of embryo sac development. We have not been able to obtain developmental stages representing this transitional period. The pictures (Figs. 7-9) show embryo sacs at an early cellular stage when the egg apparatus and antipodals are differentiating. We are not certain in what sequence these micrographs should be placed in order to yield a correct succession of developmental events. It appears that the embryo sac in Fig. 8 is more highly differentiated than the one in Fig. 7. If this assumption is correct, there would be two vacuolisation stages within the central cell during cellular embryo sac development. At the beginning of the cellular stage the vacuole present in a preceding nucleate stage is replaced by cytoplasm, which gives the whole structure an extremely compact and dense appearance. This stage is marked in the central cell by the presence of small ingrowths on the entire surface of the cell wall which borders the nucellar cells. (Fig. 36). These ingrowth disappear when the embryo sac is increasing except from the region at the level of egg apparatus (Fig. 29). The beginning of egg apparatus differentiation coincides with the presence of a large vacuole in the central cell. By the time the apparatus consists of elongated cells, the central vacuole disappears and the fusion of polar nuclei takes place (Fig. 33). The resulting nucleus is situated immediately beneath the egg apparatus (Fig. 34).

The cytoplasm of the central cell with fusing nuclei contains a large amount of twisted and whorled rough ER cisternae, mitochondria, plastids and active dicyosomes producing vesicles filled with some granular material (Figs. 35, 38). In the later stage the cytoplasm becomes vacuolated. The-

re still are mitochondria and variously shaped plastids, but very few ER cisternae and no appreciable number of active dictyosomes.

SYNERGIDS

Egg apparatus cells originally do not show any structural differences in their cytoplasm. In the further development, the cells elongate and begin to form large vacuoles; synergids in the chalazal part, the egg in the middle. Then their cytoplasm displays striking structural differences. The most obvious one is an extensive ER in synergids contrasting with almost complete lack of it in the egg. In the earlier period mostly wide, long cisternae lie along the main axis of the cell (Figs. 26, 27). All cisternae have fairly dense contents and are covered by ribosomes. The sides of some cisternae are closely pressed to the cell membrane and the wall. Their surfaces which are in contact with the cell membrane seem to be without ribosomes; therefore, these cisternae can be described as asymmetrical. In the later stage all ER cisternae are transformed into a frothy mass of slightly elongated and vesicular fragments preponderantly without visible contents (Fig. 28).

During the stage of long ER cisternae, there are numerous osmiophilic droplets indented into the electron translucent walls of the synergids and into the filiform apparatus. These droplets are very similar in shape and osmium affinity to the droplets seen at the walls of the nuclear embryo sac. In the later stage the synergid walls and filiform apparatus are homogeneusly translucent, devoid of any osmiophilic inclusions. The chalazal portion of the wall is thinned down in a similar fashion like the chalazal wall of the egg cell.

THE EGG CELL

At the time of extensive ER development in synergids, the cytoplasm of the egg remains almost without ER cisternae. The upper part of the egg is filled with homogeneous, vacuolizing cytoplasm (Fig. 32) while most of the mitochondria and plastids are scattered in the micropylar part of the cell where the nucleus is situated. Optically dense plastids are often elongated, some in the shape of cup bodies (Fig. 34). Such elongated and cupshaped plastids are characteristic of all stages of embryo sac development beginning from the meiocyte.

The wall of the micropylar part of the maturing egg is reduced to a row of electronlucent spaces joined by an osmiophilic layer assumingly formed by the glued cytoplasmic membranes of the egg and central cell (Fig. 34). These translucent parts of the cell border look like local dilutions of the adjoining cell membranes. The micropylar portion of the fertilized egg wall develops labyrinth ingrowths (Fig. 37).

DISCUSSION

During the development of the *Stellaria media* megagametophyte the ultrastructure of the cytoplasm, the nucleus and the cell wall undergoes changes similar to those described in several other plants. The callose layer, charasteristic for many species (Rodkiewicz 1970), is formed around the meiotic cells; the filiform apparatus is set up in synergids (Fig. 31) and the reduction of chalazal walls in the egg apparatus takes place similarly as has been described in some plants (ref. Jensen 1972, Fougère-Rifot 1981 and further ref., Bednara 1977, Wilms 1981 a, b,). Other changes are repeated in different stages. In this respect the cyclic alteration of endoplasmic reticulum in the nucleate embryo sac and the synergids seems to be of particular interest.

The presence of abundant rough ER in nucleate embryo sacs of *Epilobium* was discussed by Bednara (1977) and *Zea* (Russell 1979). *Stellaria* intermitotic nucleate embryo sacs contain either a number of ER cisternae or area almost without them. This suggests a tentative sequence of events for formation and degradation (possibly by vesiculation) during the two and four nucleate stages of embryo sac development (Fig. 39) where the ER develops in much greater abundance. Synergid elongated ER cisternae dilate and then fragment into vesicles as it occurs in cotton (Jensen 1965). Both in the nucleate embryo sac and in the synergids some long ER cisternae come in close contact with the cell walls. A similar relation between internal walls of the embryo sac and ER cisternae was seen in some other plants (Fougère-Rifot 1981).

The occurrence of long ER cisternae coincides with the presence of osmiophilic droplets which mostly line the embryo sac and synergid walls. Similar droplets have been reported in both the synergids and central cell of *Aquilegia vulgaris* embryo sac (Fougère-Rifot 1978). She considers these droplets to be lipid bodies and suggests that they are transported from cell to cell through plasmodesmata. From our data we may assume, however, another possible significance regarding osmiophilic droplets.

Micrographs seem to show that when certain cisternae are pressed to the cell wall they contain osmiophilic droplets and that similar droplets occupy positions both internal and external to the cell membrane. This distribution of droplets suggests that they may be incorporated into the wall. The transport of droplets through the embryo sac wall devoid of plasmodesmata seems less likely.

An association of osmiophilic droplets with the sporoderm formation in a variety of pollen grains has been reported (Vasil and Aldrich 1970, Dickinson 1976, Rodriguez-Garcia 1978). Thus, it seems reasonable to hypothesize that the osmiophilic droplets may be involved in the deposition of substances within the wall of the nucleate embryo sac and synergids

in *Stellaria*. This deposition of material, however, does not change the optical properties of the walls which remain translucent for electrons.

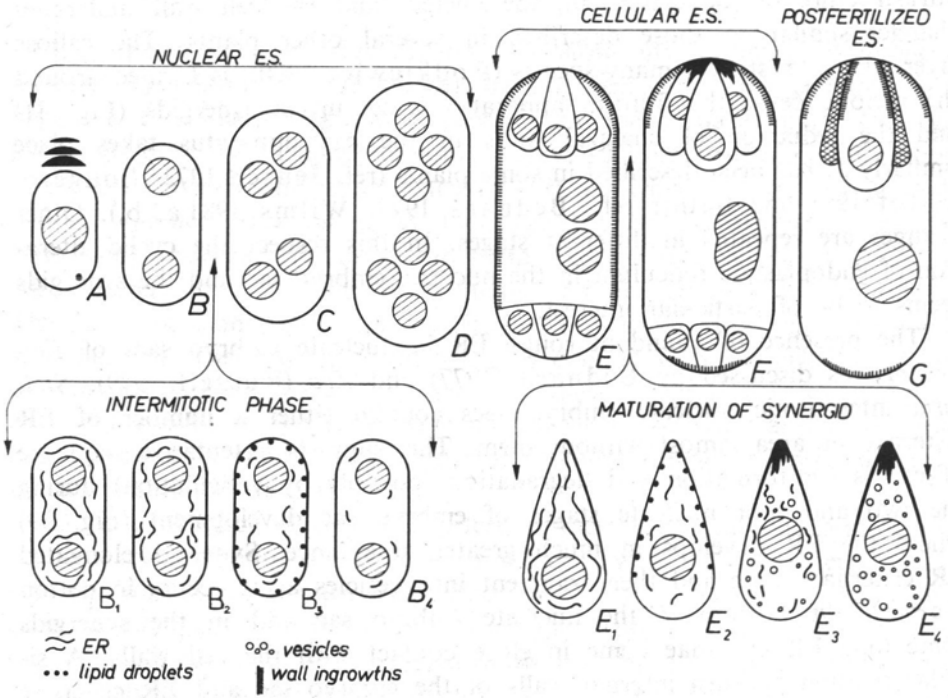


Fig. 39. Diagram of possible developmental stages of endoplasmic reticulm, osmiophilic droplets and wall ingrowths during megagametogenesis in *Stellaria media*. A-D—one to eight nucleate stages of embryo sac. E-G—cellular and fertilized embryo sac, B_1-B_4—stages between B and C; ER and osmiophilic droplets presented. E_1-E_4—stages between E and F. Similar cytoplasmic changes to those marked B_1-B_4 occur also between stages of four and eight nucleate embryo sac

Wall ingrowths have been seen in the mature embryo sacs of several plants (Diboll and Larson 1966, Gori and Sarfatti 1970, Newcomb and Steeves 1971, Mogensen 1972, Fougère-Rifot 1981). In the *Stellaria* cellular embryo sac we assume the developmental sequence presented in Fig. 39. It appears that wall ingrowths formation occurs in the central cell during the early cellular stage of the embryo sac. During the subsequent growth of the embryo sac, most of the side walls lose their ingrowths. They remain only in the micropylar part of the central cell and on the chalazal walls of the antipodals. When the zygote is formed, ingrowths develop within the micropylar portion of its wall. The localized occurrence of the wall ingrowths suggests the existence of regional differences in the physiological function of the embryo sac walls.

Acknowledgement

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Kalozowy i osmofilny materiał odkłada się do ścian komórkowych rozwijającego się grametofitu żeńskiego Stellaria

Streszczenie

Obfite szorstkie retikulum endoplazmatyczne rozwija się i znika podczas intermiotycznych stadiów w dwu i czterojądrowym woreczku zalążkowym oraz w synergidach. Cysterny ER w synergidach przekształcają się w masę pęcherzyków. Pewna liczba długich cystern ER przylega do ściany komórkowej intermitotycznego woreczka zalążkowego i do ścian młodych synergid. W tym czasie liczne osmofine kropelki przedostają się przez błonę komórkową i są prawdopodobnie wbudowywane do ściany komórkowej.