Intercellular relations and wall structures in mature embryo sacs of four species of angiosperms

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Abstract

During the maturation process of an ovule, the internal walls of the embryo sac becomes gradually thinner from the poles to the central cell. The thinning process results in an association of two plasma membranes with no polysaccharide structure between them. The presence of one disymmetrical ER cistern along the walls during the thinning process permits to anticipate a turn-over of carbohydrates.

MATERIAL AND METHODS

The mature embryo sacs of *Aquilegia vulgaris*, *Eschscholtzia californica*, *Chelidonium majus* and *Raphanus sativus* were observed and particularly the wall structures, but only the internal walls are investigated in this paper.

For electron microscopy, the ovules were fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer — pH 7.2 for 2 hr at 4°C. They were then washed and postfixed in 1% osmium tetroxide. Some sections were treated by Thiéry's method (PATg) (1967) for polysaccharides.

RESULTS

Internal walls at the chalazal pole

The walls separating the antipodal cells undergo diverse developments.

In *Aquilegia vulgaris*, the young antipodal cells are separated by walls from 0.5 to 0.7 μm thick, with a fairly dense middle lamella separating two slender fibrillar parietal layers (Fig. 4). Numerous plasmodesmata can be seen along these walls. This structure remains until the two polar nuclei, in the central cell, are fused immediately before fertilization. At that stage, two changes of the antipodal cells are noti-

- Fig. 1. *Eschscholtzia* — PATAg test. Antipodal tip in contact with the central cell before polar fusion. Note reduced walls
- Fig. 2. *Eschscholtzia* — Antipodal tip in contact with central cell while polar fusion is in process. The arrows point to a thin layer representing the parietal part
- Fig. 3. *Aquilegia* — Antipodal tip before polar fusion. The walls have become simplified. A layer of disymmetrical ER is along the plasma membrane of the central cell. Dilated swollen portions of ER are linked with the plasma membrane of the antipodal cell. The thick arrows point to the plasma membrane and ER
- Fig. 4. *Aquilegia* — Polar nuclei are coming closer. The parietal reduction process is not as advanced as in Fig. 3. A layer of commonly found ER is linked with the plasma membrane and losing its ribosomes on one side
- Fig. 5. *Aquilegia* — Link between central cell and antipodal cell following polar fusion. Only two plasma membranes are present
- Fig. 6. *Aquilegia* — Intersynergid walls before polar fusion. There are a few parietal nodules left in the zones where the plasma membranes were brought in contact (arrows). The wall reduction process may last a long time
- Fig. 7. *Aquilegia* — PATAg reaction. Link between synergid and central cell. The polysaccharide portion is reduced between the plasma membranes
- Fig. 8. *Aquilegia* — Following polar fusion. The two plasma membranes are facing each other and can be close to each other (0.07 μm in some zones near the antipodal tip)
- Fig. 9. *Chelidonium* — Two layers giving positive reaction with PATAg (arrows) remain parallel and separate between the antipodals
- Fig. 10. *Chelidonium* — The two interantipodal parietal layers (arrows) are drifting apart at the base; one of the layers is the starting point for basal parietal projections
- Abbreviations: A — antipodal cell, af — filiform apparatus, CC — central cell, er — rough endoplasmic reticulum, er d — disymmetrical endoplasmic reticulum, G — Golgi apparatus, N — nucleus, Nu — nucellus (squashed nucellar cells), p — plasma membrane, pd — plasmodesmata, pp — wall ingrowths, S — synergid, vg — Golgi vesicle.
- Line on micrograph = 1 micron (μm)

ced: their nuclei start dividing amitotically and the wall projections at the base of the antipodal cells increase.

When the fusion of polar nuclei is completed, the antipodal wall is considerably thinner (0.1 μm thick). In the upper parts of the cell, the thinning process is irregularly distributed so that thin and thicker areas can be found alternatively, giving the wall the aspect of a pearl necklace. The middle area still gives a highly positive reaction in the PATAg test. At the tip of the antipodal cells, in contact with the central cell, the wall layer is so to speak "melted" and two plasma membranes are brought together (Figs 5, 8).

At maturation of the embryo sac, wall modifications are still under way from the base to the tip of the antipodal cells. The thinning process spreads to the entire wall except in the chalazal area, near the wall projections in contact with the nucellus. In that area, the intricate,

Fig. 11. *Eschscholtzia* — Micropylar tip of synergid during fusion of the polar nuclei. The walls are gradually getting thinner. The thick arrow points to the level where the PATAg positive layers fuse

Fig. 12. *Eschscholtzia* — Link between synergid and central cell when polar fusion is over. The plasma membranes (arrows) are facing each other and are still accompanied by an disymmetrical ER cistern in the central cell

Fig. 13. *Eschscholtzia* — Link between synergid and central cell during the fusion of the polar nuclei. A PATAg positive layer remains between the two plasma membranes. Disymmetrical ER along the plasma membrane of the central cell

Fig. 14. *Eschscholtzia* — Link between egg and central cell during fusion of the polar nuclei. Layer remnants between the plasma membranes

Fig. 15. *Chelidonium* — PATAg reaction. Link between synergid and central cell in the mature ovule. The distance between the plasma membranes varies

Fig. 16. *Chelidonium*. The mature ovule, two cisterns of asymmetrical ER along the plasma membrane in the central cell and synergid (ribosomes are arrowed)

Fig. 17. *Raphanus* — Micropylar tip near the filiform apparatus before fertilization. The wall is reduced to a single PATAg positive layer (arrows) (see Fig. 11)

Fig. 18. *Raphanus* — PATAg reaction. Same section as in Fig. 17 on level with the base of a synergid linked to the central cell. A few parietal nodules are still present

Abbreviations as in Plate I.

Line on micrograph = 1 micron (μm).

fibrillar structure gives a positive reaction with PATAg with large periplasmic areas where polysaccharide polymerisation occurs. The inter-antipodal walls become very thin (below $0.1 \mu\text{m}$) with no fibrils. The cells are bordered by the plasma membrane separated by a thin layer slightly positive PATAg test and slightly granular with no plasmodesmata.

In *Eschscholtzia californica*, the development is simpler. The antipodal cells are considerably smaller with a shorter life span and their features are less marked; wall ingrowths, for instance, are less striking. The antipodal wall is not highly modified and remains homogeneous. During the whole maturation process of the embryo sac, the antipodal walls are thin, reduced to one or two layers slightly thicker than a cell membrane (Figs 1, 2). They can even disappear and then the antipodals and central cell are only separated by locally swollen sinuous plasma membranes. In the zones where the antipodals are in contact with the nucleus, the progression of the layers can be followed and its continuity underneath the wall ingrowths can be observed. It is difficult to use the word "wall" in this case. The structures are so subtle that it is hard to tell the difference between the wall and the middle lamella.

In *Chelidonium majus*, wall structures are similar but there is no fusion of the layers and they remain parallel on the entire cell surface (Figs 9-10). Likewise, these layers give a positive PATAg reaction. As

in the previous species, wall ingrowths are found on the polysaccharidic layers (Fig. 10).

Internal walls at the micropylar pole

The walls of micropylar cells follow two concomitant development patterns: one is dependent on the maturation stage of the embryo sac, the other, on the location of the wall. In all four species, the tip of the synergids is occupied by a filiform apparatus.

In *Aquilegia vulgaris*, at the time preceding the fusion of polar nuclei, the walls of the three micropylar cells resemble the antipodal walls. All walls inside the embryo sac are similar at this stage. Their structure is relatively simple; however, a middle lamella wall layers and numerous plasmodesmata can be traced. The plasma membrane shows protuberances with swellings where cytoplasmic vesicles and periplasmic fibrils meet. Near the micropylar tip, the intersynergid wall is more complex; its complexity is due to the proximity of the filiform apparatus. Thus, the short wall ingrowths which appear are but an extension of this apparatus.

At maturity, the wall structure is reduced at the site distant from the micropylar tip. The walls become gradually simplified in structure and they also get thinner. In some zones, the reduction is delayed, leaving parietal nodules separated by zones where the plasma membranes are close together (Fig. 6). Between the egg and central cell, the wall can be $0.03\text{ }\mu\text{m}$ thick, and shows no positive reaction with PATAg; a few thin fibrils can be traced between the plasma membranes (Fig. 7).

In *Eschscholtzia californica*, during the phase where polar nuclei are diametrically opposite, the walls of the micropylar cells are thin and reduced to two parallel layers between the plasma membranes which are positive after PATAg staining. They are similar to those at the chalazal pole at the same stage. Later, the structure is even more reduced and there only remains a middle PATAg positive lamella. The periplasm is rather narrow (Fig. 13). This middle lamella results from the fusion of the two original layers which are still present near the micropylar tip of the synergids (Fig. 14).

In *Chelidonium majus*, at maturation, the micropylar cells are surrounded by plasma membranes only (Figs 15-16).

In *Raphanus sativus*, the intersynergidal wall becomes thinner near the filiform apparatus and there is only one layer left. The layer becomes thinner towards the chalazal pole (Fig. 17). Parts of the layer still remain in the chalazal portion (Fig. 18). Synergids from this species are elongated cells ($60\text{ }\mu\text{m}$ long) occupying the greatest part of the embryo sac.

Relation between walls and rough endoplasmic reticulum of *Aquilegia vulgaris*, *Eschscholtzia californica* and *Chelidonium majus*

In most cases, while the wall is getting thinner, in all stages of embryo sac maturation, only one specific ER cistern can be found along the internal walls of the embryo sac cells. This ER shows a specific structure: it only bears ribosomes on the membrane lying farthest from the wall, while the membrane sticking close to the plasma membrane is bare (Figs 3, 4, 12, 13, 16). The cistern runs along the wall and extends into the ER network (Fig. 4). Many cases of "disymmetrical ER", smooth on one side and rough on the other, can be observed during the parietal thinning process. They are not present during cell wall formation, nor when the cellulose components have disappeared. Disymmetric ER was not observed in the *Raphanus* embryo sac.

DISCUSSION

The data presented show some development of the internal embryo sac walls in our species. They show analogies in development patterns and similar parietal systems in the micropylar and antipodal cells of each species. The specific features which have been exposed show that, like any other component, the walls are well integrated into the structure of the female gametophyte with its micropylar and chalazal units. These two units have strongly marked individual functions and the central cell of the female gametophyte acts as a buffer between the two poles. They thus throw an interesting light on the function of various cellular elements of the embryo sac development.

At prematurity, numerous plasmodesmata connect the similar cells of one pole. They are less numerous in contact with the central cell. They indicate the physiological individuality of both of these units and the existence of an exchange between the antipodal cells and the synergids. Many authors think that the antipodal cells have a nutritional role (Vazart, 1969; Rifot, 1973), the synergids are believed to exert a secretory activity (Godineau, 1969; Fougère-Rifot, 1975; Dumas, 1978); or are involved in the fertilization process (Jensen, 1965a; Vazart, Vazart, 1966). According to Jensen (1965a) "the synergids are active and they are involved in absorption, storage and transport of compounds from the nucellus".

The most important phenomenon in each species under study and especially in *Aquilegia* is that time and space have a part to play in the evolution of the walls: they get thinner from the poles to central cell — the changes in structure occur from prematuration until maturity is reached. The cells are subjected to a local simplification of their typical

cell walls. Eventually, the adjoining plasma membranes are the only elements present in the zones near the central cell. Intermediary stages occur during this evolution. Many species have synergids and egg cells the walls of which undergo gradual thinning (*Torenia fournieri*, van der Pluijm, 1964; cotton, Jensen, 1965a-b; *Crepis*, Godineau, 1966-1973; flax, Vazart, Vazart, 1965-1966; flax, Vazart, 1969; maize, Diboll, Larson, 1966; maize, Diboll, 1968; *Aquilegia vulgaris*, *Clematis vitalba*, *Ranunculus acer*, Rifot, 1969; flax, d'Alascio-Deschamps, 1973). According to Newcomb (1973) in *Helianthus*: "The synergid-central cell boundary consists of two plasma membranes separated in regions by pockets of wall material". The egg wall may be thicker than that of the synergid (*Crepis*, Godineau, 1966); before fertilization in *Capsella* the synergid wall is thicker at the micropylar than at the chalazal pole, but there still remains a well with a positive PAS reaction (Schulz, Jensen, 1968b). "The synergids of *Capsella* then, seem unique in possessing a chalazal wall". Following fertilization, parts of the walls disappear and "there is a gradual mixing of the synergid and central cytoplasm" (Schulz, Jensen, 1968a). In 1969, Bouillot found that *Juglans regia* gave a negative PAS reaction in its micropylar zone. Yet, Mogensen (1972) shows that a wall was gradually formed around the synergids and egg of *Quercus gambelii*. Wall formation starts in the phase preceding fertilization and continues in the phase following fertilization.

The thinning of internal walls strengthens the homogeneity of the sac. Markedly different are the walls in the nucellus or the integuments which are thick. They form a rigid framework which is crushed under the heavy internal pressure from the embryo sac. The collapse of the frame is also associated with the lower rate of turgor in nucellar or integumental cells the cytoplasm of which are gradually disintegrated. In this way, a rather heavy wall is built up around the embryo sac, the piled up walls form a polysaccharide supply all around it. In two species of *Euphorbia*, Gori and Sarfatti (1970) pointed out that "le sac embryonnaire est entouré par une épaisse "paroi" de matériel pectocellulosique. La partie externe de cette "paroi" présente une stratification naissant de plusieurs parois des cellules du nucelle désormais vidées et peu à peu pressées par le sac embryonnaire grandissant".

In the mature embryo sac, polysaccharides are found in a few privileged sites, as in the amyloplasts of the central cell, the filiform apparatus, the wall projections at the base of the antipodal cells and the minor projections of the sac walls in contact with the nucellus (Fougère-Rifot, 1979b). In the synergids also, numerous Golgi vesicles are present (Fougère-Rifot, 1979a). During synthesis of polysaccharides and their accumulation in some structures, the amount

of polysaccharides decreases in the internal walls of the embryo sac. What relation exists between these two phenomena? The decrease of the wall polysaccharides which practically disappear, raises a problem as to how they are transported. During the wall thinning process, a layer of disymmetrical ER lies along the walls. The situation can be compared with that in pancreatic acinar cells of bats (Fawcett, 1967). In these cells where regular layers of ER cisterns are numerous, a row of ribosomes having no link with an apparent membrane structure follows the plasma membrane, the distance between them being approximately similar to the thickness of an ER cistern. During the cell growth process in the mung bean hypocotyl, Prat et al. (1977) noticed: "significant associations between sheets of endoplasmic reticulum and plasmalemma; typically, the endoplasmic reticulum is rough towards the cytoplasm and smooth towards the plasmalemma". The reticulum observed in the embryo sac seems a very similar structure. The organization in the embryo sac where an intense glycide metabolism occurs points to a possible transfer of simple glycodes from the walls to the ER, their transport through the reticulum network, integration into the Golgi apparatus, transformation and transfer towards the filiform apparatus and the wall projections. Such a progression of the carbohydrates tends to show that there is a turn-over of glycide molecules in differentiated systems of the embryo sacs.

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