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Callose in Cell Walls during Megasporogenesis in Angiosperms

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Summary. Callose was detected by fluorescence microscopy in megasporogenesis in all investigated species with mono- and bisporic embryo-sac development. Callose occurs first in the meiotic prophase in the chalazal part of the megasporocyte wall and by the first meiotic metaphase the whole cell is enveloped in a callose-containing wall. Later, there is a marked decrease of callose fluorescence, usually at the chalazal end of the megasporocyte. In *Oenothera*, where the micropylar megaspore is active, decrease of fluorescence takes place at the micropylar pole of the megasporocyte. Callose appears centrifugally in the cell plates forming eventually the walls dividing the megaspores. It disappears from the walls of the megaspores during degeneration and differentiation.

Introduction

At the end of the 19th century Mangin (1889) described callose walls in microsporogenesis (ref. Eschrich, 1956). Young meiocytes in the anther are connected by thick cytoplasmic strands which disappear at the end of the first meiotic prophase when each meiocyte is coated by a special callose wall; this wall is dissolved later in the tetrad stage (Heslop-Harrison, 1964; Weiling, 1965). According to Heslop-Harrison (1964, 1966) the most reasonable interpretation of the function of the callose wall is that it acts as a molecular filter, permitting the passage into the spores of basal nutrients but excluding larger molecules. In this way a high degree of isolation of each meiotic cell is brought about.

Callose was noted in megasporogenesis of one gymnospermous species (De Sloover, 1961; Waterkeyn, 1961) and found in megasporogenesis of several species of angiosperms (Rodkiewicz, 1967, 1968; Rodkiewicz and Górska-Brylass, 1967, 1968). The events of callose appearance and disappearance in megasporogenesis of angiosperms were investigated further. Callose occurs in the cell wall around the megasporocyte at the meiotic prophase and later in the transverse walls of dyads and tetrads. It eventually disappears from the tetrad when an active megaspore differentiates into the embryo-sac. Callose was observed in species with the monosporic type of female gametophyte development but not with the tetrasporic type.

Table. *List of Investigated Species and Callose Responses Observed*

Type of embryo-sac development: m = monosporic. b = bisporic, t = tetrasporic.

+ = strong fluorescence, △ = weak fluorescence, − = negative.

Family	Species	Type of development	Fluorescence	
			Cross walls	Side walls
Campanulaceae	*Campanula latifolia* L.	m	+	△
	Campanula persicifolia L.	m	+	△
	Campanula rapunculoides L.	m	+	△
Caryophyllaceae	*Agrostemma githago* L.	m	+	+
	Melandrium album (Mill. Garcke)	m	+	+
	Saponaria officinalis L.	m	+	+
	Stellaria graminea L.	m	+	+
	Stellaria holostea L.	m	+	+
Euphorbiaceae	*Ricinus communis* L.	m	+	+
Liliaceae	*Allium cepa* L.	b	+	△
	Funkia umbellata Spreng.	m	+	+
	Galtonia candicans Dcne.	m	+	+
	Hemerocallis fulva L.	m	+	+
	Lilium candidum L.	t	−	−
	Lilium regale Wils.	t	−	−
	Polygonatum officinale All.	m	+	+
	Tulipa spec. (cultivars)	t	−	−
Lobeliaceae	*Laurentia longiflora* L. Emdl.	m	+	+
Onagraceae	*Epilobium hirsutum* L.	m	+	+
	Fuchsia corymbiflora Ruiz. et Pav.	m	+	+
	Oenothera biennis L.	m	+	+
Orchidaceae	*Epipactis latifolia* L. All.	m	+	+
	Orchis maculata L.	m	+	+
	Platanthera bifolia L. Rich.	m	+	+
Papaveraceae	*Eschscholtzia californica* Cham.	m	+	△
	Papaver glaucum Boiss. et Hauskn.	m	+	△
	Papaver orientale L.	m	+	△
	Papaver atlanticum Ball.	m	+	△
Pirolaceae	*Monotropa hypopitys* L.	m	+	△
Primulaceae	*Primula officinalis* L. Hill.	m	+	+
Ranunculaceae	*Caltha palustris* L.	m	+	+
	Delphinium hybridum (cultivar)	m	+	+
Saxifragaceae	*Ribes aureum* Pursh.	m	+	+

Table (Continued)

Family	Species	Type of development	Fluorescence	
			Cross walls	Side walls
Scrophulariaceae	*Antirrhinum majus* L.	m	+	+
	Digitalis ambigua Murr.	m	+	+
	Gratiola officinalis L.	m	+	+
Solanaceae	*Atropa belladonna* L.	m	+	+
	Capsicum annuum L.	m	+	+
	Datura ferox L.	m	+	+
	Hyoscyamus albus L.	m	+	+
	Hyoscyamus niger L.	m	+	+
	Nicotiana tabacum L.	m	+	+
	Solanum tuberosum L.	m	+	+

Materials and Methods

Ovaries of 43 species belonging to 14 families (see Table) were fixed in alcohol-acetic acid (3:1). Before fixation parts of the ovary wall were removed to ensure quicker penetration of the fixative into the ovules. Care was taken not to damage the ovules in order to avoid formation of wound callose. According to Engelman (1965) callose in stems is accumulated in increased amounts in response to wounding; however, this occurred only 5 min or more after wounding and only within a distance of about 15 sieve elements from the wound. The parenchyma callose reaction is confined to the wound border and this process, as recorded by Currier (1957), is very rapid. Although the possibility of formation of wound callose in megasporogenesis has not been excluded, it is unlikly, however, that the callose observed in megaspores was wound callose. There was no wound callose in any somatic cell of the ovule, and there was none in the coenomegaspores of tetrasporic species which were fixed according to the same procedure as monosporic ovules.

Fixed ovules were hydrolized in 1N HCl for 5—10 min, then rinsed in water. Callose was identified in ovule squashes by the callose Aniline-blue fluorescence method and by Resorcine-blue staining (Eschrich and Currier, 1964).

Results

Callose was detected in megasporogenesis in all 39 species with the monosporic type of embryo sac development and in the one investigated species with the bisporic type; in contrast, callose was not seen in 3 species with the tetrasporic type of development (see Table). Most of the species exhibit strong callose fluorescence both in the side walls of the megaspores and even stronger in the transverse walls. Some species, however, display weak callose fluorescence in megasporocytes and in the side walls of later stages, but have strongly fluorescing transverse walls (see list).

In young ovules premeiotic and early meiotic megasporocytes are surrounded by a thin primary wall containing polysaccharides which

give a positive periodic-acid Schiff (PAS) reaction. Later, when the megasporocyte reaches the pachytene or diakinesis stage, its cell wall begins to show callose fluorescence. In the late prophase and especially in the first metaphase, the whole meiocyte is surrounded by a wall containing callose. Callose penetrates the primary wall and sometimes forms small granules protruding into the cell. Callose in the primary wall is visible after Resorcine-blue staining. At this stage the meiocytes are the only cells in the ovules which show callose fluorescence. After the first and the second meiotic divisions the dyads and tetrads are separated by cell walls rich in callose. As early as by the time of formation of the transverse walls, callose appears centrifugally in the cell plate, before the division of the cell is accomplished (Figs. 1f, 5b).

The pictures in Fig. 1a—d show *Epipactis* and *Platanthera* meiocytes in the light microscope and, underneath, Fig. 1e—h the same stages in the fluorescence microscope, after Aniline-blue staining. Strong fluorescence of the chalazal pole is visible, as well as fluorescence of the cell plate at the late first telophase. In the dyad the chalazal pole shows very slight fluorescence, which does not come off distinctly in the picture; in the linear tetrad, callose occurs in the transverse walls and at both apices. A strong fluorescence is also shown by the upper parts of the side walls of the active megaspore.

The pictures in Fig. 2 show the megasporogenesis in *Laurentia*, a species with the *Polygonum* type of embryo-sac development (monosporic, 8-nucleate). Megasporocytes at the middle of the first meiotic prophase fluoresce strongly at the chalazal pole and much more weakly at the micropylar end. The intensity of fluorescence changes in the late first prophase and metaphase. It decreases at the chalazal end whereas the large segment of the wall above the chalazal pole displays stronger fluorescence; this latter persists throughout the dyad and the beginning of the tetrad stage. In the tetrad stage the chalazal end again accumulates callose. The very young tetrad (Fig. 2d) is shown just when two new walls are laid down. They are becoming thicker in the later stage when the chalazal megaspore, being the mother cell of the embryo sac, loses callose in the process of differentiation.

The pictures in Fig. 3a show a meiocyte of *Gratiola* (*Polygonum* type) with the chalazal wall already devoid of callose. In the triad stage the chalazal megaspore has a partly fluorescing wall, and the chalazal end is visible due to a small callose grain present. Triads are formed after the asynchronous second meiotic division which occurs frequently in some species (in the *Polygonum* type, the chalazal cell is dividing first). (Figs. 3b, c, 4c). A triad of an orchid, *Epipactis*, has slanting walls and the tetrad often shows a peculiar arrangement of walls in the shape of an X.

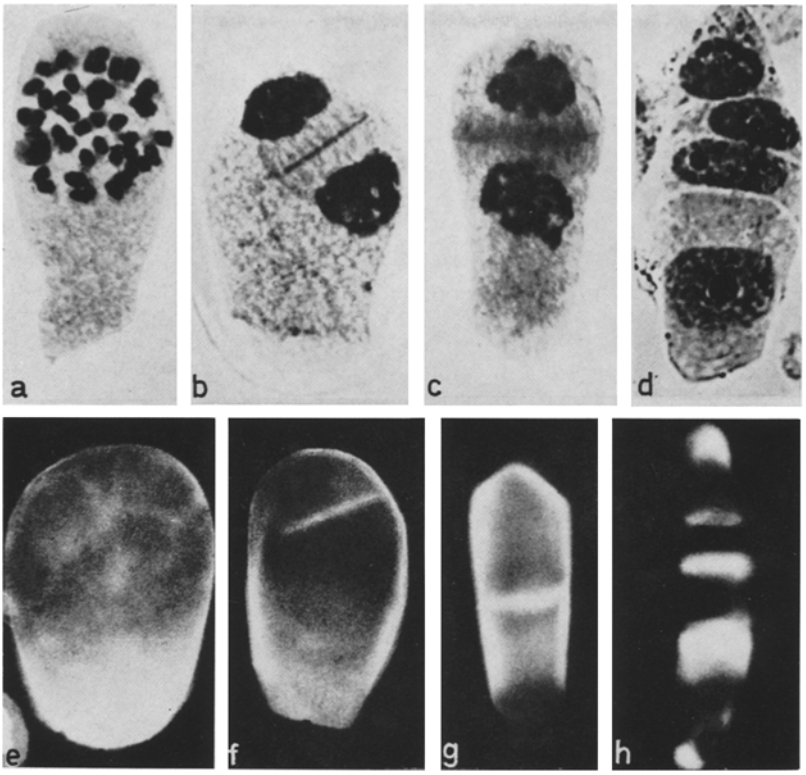

Fig. 1a—d. Megasporogenesis. a, b *Epipactis latifolia*, megasporocyte and first meiotic telophase; c, d *Platanthera bifolia*, dyad and tetrad; e—h the same stages of megasporogenesis seen in the fluorescence microscope after Aniline-blue staining for callose (×1,000)

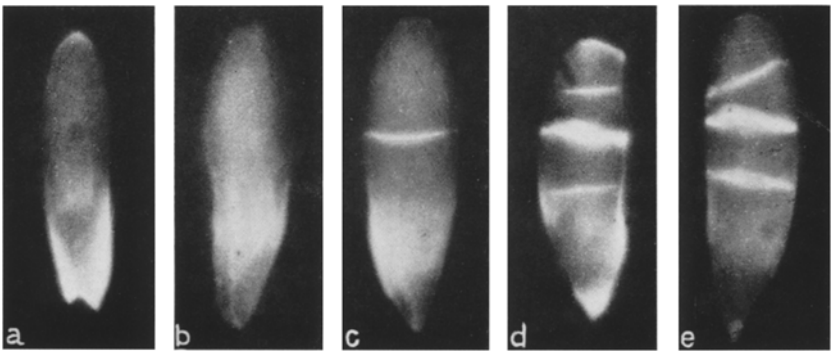

Fig. 2a—e. Callose fluorescence in megasporogenesis in *Laurentia longiflora*. a Megasporocyte in the middle of the first meiotic prophase; b megasporocyte at the end of the first meiotic prophase; c dyad; d young tetrad just after formation of transverse walls; e older tetrad with the chalazal megaspore showing very weak fluorescence (×1,200)

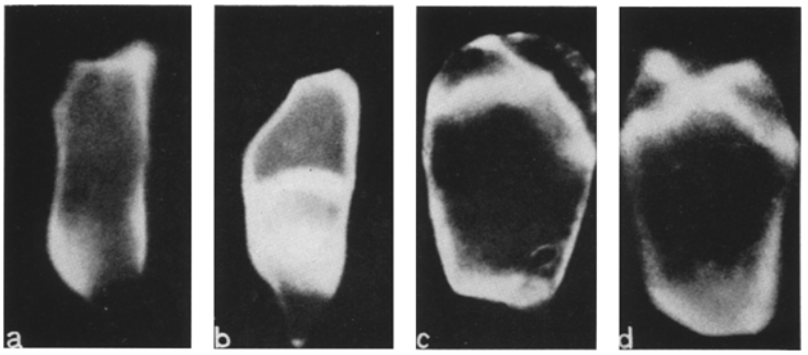

Fig. 3a—d. Callose fluorescence in cell walls during megasporogenesis. a b, Megasporocyte and triad of *Gratiola officinalis*; c, d triad and tetrad of *Epipactis latifolia* (×1,000)

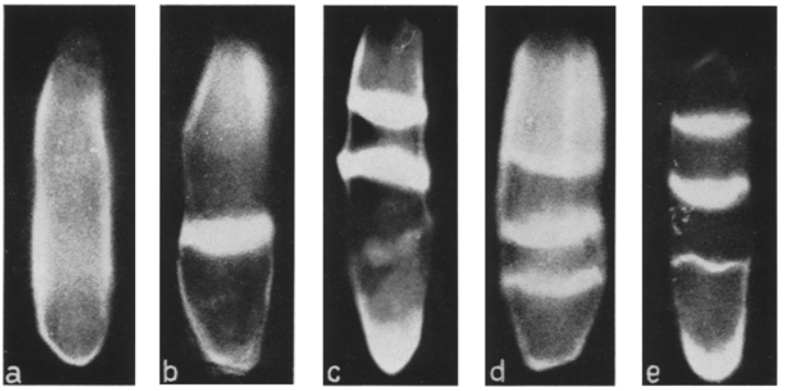

Fig. 4a—e. Megasporogenesis in *Oenothera biennis*. a Megasporocyte in late meiotic prophase with micropylar pole showing weak fluorescence; b dyad; c triad; d tetrad; e tetrad at the later stage without fluorescence in side walls or at the micropylar pole (×1,200)

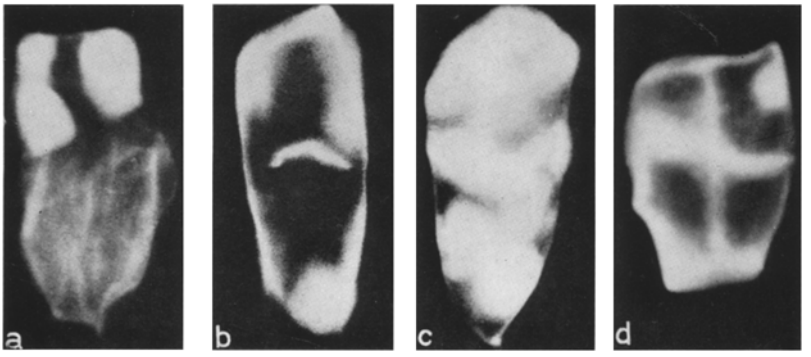

Fig. 5a—d. Callose fluorescence in *Polygonatum officinale*. a Megasporocyte with callose warts at the micropylar end; b late first meiotic telophase with a cell plate; c degenerating dyad packed with callose; d isolateral tetrad (×700)

The *Oenothera* type of development (monosporic, tetranucleate) is usually characterized by reverse polarity, i.e., the micropylar megaspore functions as embryo-sac mother cell. The *Oenothera* type is found only in the family *Onagraceae*. The reversed polarity becomes apparent at the beginning of callose appearance. Callose is laid down first in the micropylar wall of the megasporocyte. Later the whole cell is surrounded by a callose wall and, at the end of the first prophase, it is the fluorescence of the micropylar pole that undergoes a decrease (Fig. 4a). The fluorescence of this pole remains very weak during the dyad, triad and tetrad stages. At the end of the tetrad stage, the side walls of all megaspores lose fluorescence almost completely. Fluorescence persists however in the transverse walls and in the chalazal wall of the tetrad (Fig. 4d—e).

An abnormal situation, frequent in megasporogenesis of *Polygonatum* is shown in Fig. 5a, c, d. The specimens investigated grew in a garden and were almost completely sterile. In Fig. 5a, one can see a megasporocyte with large protrusions of callose at the micropylar pole; in Fig. 5b an apparently normal dyad with a fluorescing cell plate. The dyad packed with callose and the isolateral tetrad in Fig. 5c, d are presumably in a state of degeneration.

During the development of the ovule and megasporogenesis, the megaspores remain the only cells displaying strong callose fluorescence; but in species where a hypostase is formed, its cells are also fluorescing. Callose disappears entirely from the megaspores in the early development of the active megaspore and during degeneration of the three inactive ones.

Discussion

Occurrence of callose in the cell walls during megasporogenesis is a very similar phenomenon to the formation of callose-containing walls in microsporogenesis. In both processes callose appears at the first meiotic prophase, envelops the spores, and disappears in the tetrad stage.

The distribution of callose in the cell walls in megasporogenesis changes in the course of development. It always appears in the first meiotic prophase; but whereas in the *Polygonum* type of embryo-sac development callose fluorescence is found first at the chalazal pole of the meiocyte, in the *Oenothera* type it is the micopylar pole which displays the fluorescence first. Later, when the rest of the cell wall contains callose, the chalazal pole in the *Polygonum* type and the micropylar pole in the *Oenothera* type show some fluorescence, although it is at the best very weak. The different patterns of callose distribution stem probably from the fact that the megasporocytes and following generations of cells display very distinct polarity. In the *Polygonum* type the chalazal megaspore and in *Oenothera* the micropylar megaspore are active. The only exceptions showing no decrease of fluorescence at the chalazal region of

the wall are orchids (*Epipactis* and *Orchis*). Thus it may be assumed that callose is first laid down in that side of the megasporocyte wall where the active megaspore will be formed.

It seems possible that the permeability of the cell wall containing callose is less than that of cellulose cell walls. There are several indications that in microsporogenesis the formation of callose walls coincides with the drop in incorporation of radioactive compounds into RNA and proteins in the meiocytes (Taylor, 1959; Albertini, 1967; Sauter, 1967). This incorporation is again resumed after the dissolution of the callose walls. Special attention to the problem was given by Heslop-Harrison and Mackenzie (1967). In their experiments, soluble 2-^{14}C-thymidine derivatives moved freely into the meiocytes in the *Lilium* anther until the pachytene stage; subsequently, they were excluded from the pollen mother cells until dissolution of the tetrads, when the young spores again took up the tracer readily. Heslop-Harrison (1964) assumes that the expression of genetic individuality in each spore must obviously depend upon insulation from others and upon escape of all from control by the parent.

In megasporogenesis the callose-containing walls may likewise play a part in the formation of a barrier between those cells which are differentiating into megaspores, and the somatic cells of an ovule. This barrier, of which callose may be an important ingredient, brings about a temporary isolation of the differentiating cell from some correlative agent(s) present in the organ. Consequently it takes part in the events setting a meiocyte on the independent path of development leading to the formation of a female gametophyte.

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