Megasporogenesis and early megagametogenesis

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Abstract

Qualitative and quantitative data on organelle distribution during megasporogenesis and megagametogenesis were discussed. Megaspocyte and megaspore ultrastructural and cytochemical characters were related to developmental stages and embryo sac formation. The role of nucellus was considered.

SPOROGENESIS AND GAMETOGENESIS

Sporogenesis in higher plants takes place in the anther and ovule. In micro- and megasporogenesis as well as gametogenesis the sporophyte plays a dominant role. In these complex tissues sporogenesis is followed by gametogenesis, and the heterotrophic micro- and megagametophyte produce more or less reduced gametes.

In general, several stages of sporogenesis can be distinguished in higher plants. Its induction in the form of meiosis, is most often accompanied by a protective callose wall, and results in the separation of spores. During microsporogenesis, wall formation and production of storage substances in relation to the future transport and quiescent period are involved. In megasporogenesis some characteristics such as exine differentiation, dehiscence mechanism are absent. The microgametophyte differentiates to form a tube for the transport of the sperm cells. However, there are great differences in the shedding stage of pollen. Some pollen have two sperm cells and an elaborate germination apparatus. But most of them have a generative cell, and germinate slowly. Many differences also exist in the quality and quantity of metabolites. The same is true about megagametogenesis of which different types of development and final stages are well known.

Sporogenesis and gametogenesis are highly related to the surrounding tissues. In the anther the tapetum, middle layer, endothecium and epidermis form a layered structure. In the ovule a nucellar tissue covered by an epidermis has a more homogeneous composition. A difference

in tissue composition can, however, be noted as a more elaborated chalazal part in some species. This sporophytic-gametophytic interaction is a determining factor in sporo- and gametogenesis.

QUALITATIVE CHANGES IN CELL ORGANELLES DURING MEGASPORO- AND EARLY MEGAGAMETOGENESIS

Details of the process of megasporogenesis are well known through light microscopic and partly electron microscopic investigations. In general these studies start after the induction of sporogenesis and close ultrastructural studies during the entire development are very scanty. Because of the interaction of the developing megaspore and its surrounding tissue basic information of the whole process is necessary. The megaspore mother cell is characterized by its volume, large nucleolus, dense cytoplasm with small vacuoles and all kinds of organelles. Contact with the surrounding nucellar cells exists by means of plasmodesmata. Changes in this cell can be noticed on the onset of meiosis (De Boer-de Jue, 1978). Callose is deposited on the cell wall in several species (Rodkiewicz, 1970). Contact with the chalazal nucellar cells may however persist. After the degeneration of three megaspores this wall is broken down by the surrounding nucellar cells. Megagametogenesis starts with the storage of reserve products and formation of a large vacuole.

From this cytological study, based mainly on previous work on megasporogenesis, some events in the changes in the organelle composition can be considered (Fig. 1). The nucleus enlarges and an undulation of the nuclear membrane can be observed at zygotene. Also the increase in nucleolar size until diplotene is remarkable. The undulation of the nuclear membrane can be considered as synthesis of a new nuclear membrane necessary for future divisions. During the formation of the nuclear membrane, parts of the early membrane are used. The nucleolus has many ribonuclear protein particles which are the base for renewal of the ribosome population during metaphase I. During microsporogenesis as well as megasporogenesis the number of ribosomes decreases around zygotene and is restored again. Of this new population of ribosomes, more than a quarter will be present in the functional megaspore (Willemse, Bednara, 1979). The nuclear-cytoplasmic interactions during meiosis as described are exhibited by the nuclear membrane (Bell, 1975). A high number of pores, sacculation of the inner nuclear membrane as well as bleb formation of the outer membrane can be considered as such interactions which are related to transition to the haploid condition of the cell. But also their direct function in cell division cannot be ruled out. Since RNA transport has been demonstrated during the

Fig. 1. Quantitative data of some morphological features during megasporogenesis and megagametogenesis in *Impatiens* (I), *Allium* (A) and *Lilium* (L)

d — filled blocks represent 500 ribosomes; e — 15 polysomes per 0.6 μm^2 cytoplasm; g — filled blocks represent 7.5 dictyosomes per 150 points cytoplasm; h — the same per 50 points; l — filled blocks represent 50 mitochondria per 150 points cytoplasm; m — the same per 50 points; n — filled block represents 10 plastids per 150 points cytoplasm; o — the same per 50 points; r — filled block represents 2500 points cytoplasm, magnification 8000 $\times$; s — the same at 2500 $\times$

early tetrad stage in *Pinus* (Dickinson, Potter, 1975) all types of nuclear exchange need further examination. If RNA exchanges and ribosome renewal takes place, a possible change in the protein pattern can be expected. Exploration of the protein pattern during microsporogenesis and an analysis of polysomes should be considered in this context.

The membrane system is represented by the endoplasmic reticulum (ER), dictyosomes, vesicles and vacuoles. An excessive quantity of ER appears in *Lilium*, but normally the ER is not greatly elaborated. RER is not always present. The rate of vacuolation is very low but large vacuoles are formed in the functional megaspore. The production of dictyosome vesicles is related mostly to the cell wall formation, and callose. Many vesicles are seen and their contents show a great diversity. Mainly polysaccharides and proteins, partly as enzymes, are demonstrated (De Boer-de Jeu, 1978; Willemse, Bednara, 1979). A detailed investigation of the synthesis, transport and storage of the cellular membrane system is necessary to understand the relationship of this system with the process of megasporogenesis.

The energy supply is carried out by the mitochondria and plastids. A change in the contents of these organelles give an indication of this possible role. The decrease of the mitochondrial inner membrane expressed in the form of a low number of cristae, is very common in sporogenesis and gametogenesis. This state of low metabolic activity in the cell (Hoekstra, 1979) can be a result of the loss of cristae only when the total number of mitochondria remains constant. Plastids show a difference in thylakoid number and also in the presence of starch. Some differences perhaps relate to the low metabolic activity of the cell. Although the above mentioned differences are associated with cellular activity and storage, their more specific action can be very valuable in the characterisation of megasporogenesis. Just in the energy and storage function the process shows many specialities. Storage of amylum or lipid granules or both can be interpreted as a function for future period with less energy supply. During the storage phase, mainly before the formation of the cellular embryo sac, a food supply of the surrounding tissue is barred or blocked. No attention is paid to the structure of the ground plasm. In general this cellular component gets very little attention. Because of the complexity of sporogenesis in which the selection of one megaspore, as well as the onset of the megagametogenesis are involved, the above mentioned characteristics should be considered as a whole process and not in parts only.

A special and regulated degeneration process takes place after the first or second meiotic division. The non-functional megaspores are characterized by high density of ribosomes, appearance of lipid granules, presence of dictyosomes and many vesicles and autophagic vacuoles.

The digestion of the cell can serve as nutrition for the functional megaspore (De Boer-de Jeu, 1978). The question arises whether this degeneration depends on external factors or on a programmed process in the megasporocyte. In case of an external factor the signal for breakdown can originate from the outside and in case of an internal factor, the products formed in the cell only can be responsible.

Most studies pay attention to the presence of many ribosomes and enzyme-containing vesicles in the cell and accentuate the autolytical process. If degeneration of the megaspore is autolytic, it should have no relation with the selection of the functional megaspore. Compared with the degeneration of the nucellar cells near the micropyle, this process also depends on the growth of the megaspore as an external factor. The functional megaspore starts gametogenesis with an increase in storage products and formation of vacuoles.

QUANTITATIVE CHANGES IN CELL ORGANELLES DURING MEGASPORO- AND MEGAGAMETOGENESIS

A quantitative approach is a comparative and somewhat selective method and only the tendency is presented. In relation to a fixed area of cytoplasm and to the growth of the cell volume in general an increase is observed in the number of plastids, mitochondria — mainly before the meiotic divisions — and of vacuoles thereafter. The dictyosomes fluctuate and decrease in number after the meiotic divisions. This means that megasporogenesis starts with an increase in the number of cell organelles related to the energy supply and megagametogenesis starts with vacuolation, without an increase of the number of some organelles. Dictyosomes and ribosomes also show a less constant increase (De Boer-de Jeu, 1978; Willemse, Franssen-Verheijen, 1978) (Fig. 1). It must be emphasized that the quantitative approach must be considered in relation with the morphological changes of the organelles, their localization and cellular activity.

LOCALIZATION OF CELL ORGANELLES

During megasporogenesis the polarity of the functional megaspore assumes special importance. Such phenomena as the absence of callose on the chalazal side, the nutritive function of the chalaza, contact through plasmodesmata between the basal part of the megaspore and the polar position of some cell organelles have been noted (Willemse, Bednara, 1979).

One of the signals of cell organelle localization is the position of the nucleus. During megasporogenesis the position is mostly in the

centre of the cell. In *Lilium* too the position is central. However, if the more regular form of the cell changes this position, the widest side is chosen. A correlation exists between the shape of the cell as determined by the surrounding tissue and the position of the nucleus. Included is the possible relation to the ER. In many cases ER is concentrated around the nucleus. But this position and the interrelation are dominated by the shape of the cell, which depends on the surrounding tissue too.

An important factor in cell organelle redistribution can be the division of the nucleus. The cell organelles are located out of the spindle region and will move after the division around the two nuclei. Unequal cell division will select the amount of cytoplasm. This effect of localization in relation to cell division depends on the rate of movement of the organelles. Movement during megasporogenesis has been observed to be very slow.

Also related to nuclear division is the position of dictyosomes, which are situated close to the nuclei for the formation of the nuclear membrane and cell wall.

Another most marked position during megasporogenesis is the position of the plastids (De Boer-de Jeu, 1978; Rodkiewicz, Bednara, 1974; Jalouzot, 1971). They are present on the side of the future functional megaspore. As an internal factor it should include the self-movement of plastids, but the external factor may be the geometric factor (De Boer-de Jeu, 1978), although in that case more organelles should have a determined position. A gradient, determined by the presence of callose and basal plasmodesmata is the possible way of a polar nutritive supply (Willemse, Bednara, 1979). Indication of such a factor should have effect on other types of organelles too, especially those which are associated with assimilation such as mitochondria and plastids. However, many organic components in the ground plasm are acceptors for the nutrients too. From this point of view the presence of nutrients in the cell should constitute an at random situation because of the interaction and special relation of organelles in the nutrition. The effects of polar nutrition are probably vesicle formation in the chalazal cytoplasm of *Gasteria* and the preferential presence of vesicle with material originating from the chalazal nucellar tissue (Willemse, Bednara, 1979). Besides polarity, the formation of a megaspore takes place in a phase in which the nutritive supply is polar, but probably on a very low level which is probably responsible for the selectivity of the functioning megaspore and degeneration of others.

The cellular organisation is a problem of organelle movement, organelle interaction, organelle relationship and cellular differentiation. A cell gets, based on this kind of relations, an ordered and distinct composition. With this internal composition related to a function, the sur-

rounding tissue affects the organisation as the determination of the cell shape volume or the polar position of the cell in relation to nutrients. For megasporogenesis the organelle localization results in the future separation and formation of one functional megaspore and its differentiation into an embryo sac. An indication is given by the position of the nucleus, plastids and probably the ER. The low nutritive supply is associated with the degeneration and viability of the megaspores. The signals of renewed nutritional activity in the form of appearance of lipids, proteins and enzymes become clear at the onset of megagametogenesis. Differentiation of the megaspore and megagametophyte is also related to the surrounding tissue.

NUCELLAR TISSUE

During megasporogenesis the nucellar tissue develops simultaneously. In many reports contact of megasporocyte with nucellar cells through plasmodesmata is reported (De Boer-de Jeu, 1978; Rodkiewicz, Bednara, 1976). This contact is cut off by the callose wall, except in the chalazal part of the nucellus. Fewer similarities are found in the development of the nucellar cells and the megasporocyte. The micropylar nucellar cells start with a meristematic character and get some vacuoles. In some cases a decrease in polysomes is noted and the loss of mitochondrial cristae occurs nearly at the same time as in the megaspore; most often this means at pachytene. This means that this phenomenon can be related to a situation of less cellular activity in the entire nucellar tissue (De Boer-de Jeu, 1978). A difference exists in the contents of lipid granules and starch between the nucellar cells near the micropylar part and chalazal part of the nucellus. Near the chalazal side more storage substances can be present. In some of these cells fewer vacuoles and some strands of ER are seen (Willemse, Bednara, 1979). The nucellar tissue starts degenerating when megagametogenesis starts. The micropylar nucellar cells near the degenerated megaspore also degenerate. The chalazal nucellar cells may divide at this moment, although the rate of cell division in the nucellar tissue diminishes (Fig. 2). In this first development the heterotrophic megasporocyte begins to come into close contact with the chalazal nucellar cells. In many cases this zone has a meristematic character, a nucleus with less heterochromatin and a few small vacuoles. At this point a tissue intermediate between the transport tissue and the developing megagametophyte can be observed to differentiate. In the chalazal part of the nucellar tissue close to the megaspore the presence of different enzymes is stated; in *Gasteria* also in the intermediate zone a peroxidase reaction has been reported. After the formation of the megaspore the quantity

of the peroxidase iso-enzymes as well as the pattern of its localization are nearly constant. In the development of the nucellus the stage during the differentiation of the embryo sac, the first part of the differentiation processes are related more to the future fertilization and embryogenesis.

Fig. 2. Changes in nucellar tissue in *Gasteria*

In conclusion, the development of the embryo sac is a process, mainly elaborated by the developing megasporocyte. Until the end of megagametogenesis, the surrounding tissue maintains contact between the transport tissue and embryo sac. Its differentiation is more directed to the post-fertilization processes, except for some degeneration occurring to provide a place for the growing megagametophyte.

MICRO- AND MEGASPOROGENESIS

With the above background some resemblance can be expected in micro- and megasporogenesis just in that part in which they are independent of the surrounding tissue and less differentiated. In a totally different sporangial tissue, and with another target in differentiation, some characteristics during megasporogenesis can be found in microsporo-

genesis in *Gasteria* (Willemse, Franssen-Verheijen, 1978; Willemse, 1972). Mainly the first stages permit a comparison of these species (Fig. 3). The main changes in sporogenesis are represented with the exception of vesicles in the nuclear membrane which are observed during megasporogenesis. Although such a comparison is tentative, the conclusion can be drawn that until the formation of the young spore, the process of sporogenesis is similarly development on the surrounding tissues. During the onset of meiosis and its elaboration the tissue starts differentiating and during a part of this process the sporocyte is protected by a callose wall. However, the tissue is feeding the cell mainly during the beginning of development and later the megaspore.

Fig. 3. Subcellular and functional activities during mega- and microsporogenesis

The study of sporogenesis, however, needs elaboration, especially with regard to the complete sequence of the process, including the formation of the seed, as well as at the level of tissue interaction. Physiological aspects should be added. These first attempts emphasise the complexity of the process of developing an individual spore within a differentiating tissue.

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