Cytochemical investigations of the megasporocyte and embryo sac in *Lilium regale* at various stages of development

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ABSTRACT

During megasporocyte and embryo sac development in *Lilium regale*, characteristic changes were noted within the cytoplasm. In the course of prophase I there appear parallel systems of endoplasmic reticulum cisternae which further transform to cytoplasmic bodies built of protein and different-sized lipid inclusions. During maturation of the embryo sac the cytoplasmic bodies and lipid inclusions synthesized during prophase I undergo hydrolysis and constitute storage material utilized during maturation of the embryo sac.

INTRODUCTION

The embryo sac of *Lilium regale* develops according to the *Fritillaria* type (4-spore, 8-nucleate). Within the cytoplasm of the megasporocyte many morphological changes may be seen. Filamentous structures have been described, arranged around the megasporocyte nucleus or radially, and transforming in further development to weakly basophilic spherical bodies (Mottier, 1893; Bouin and Bouin 1898). According to Orman (1913) there are minute granular mitochondria in the megasporocyte cytoplasm and the above described granular spherical bodies contain lipids. Guillermond (1924) using Regaud's fixative found that the results obtained by the above quoted authors are artefacts produced by osmium fixatives. He considers that in the megasporocyte and embryo sac cytoplasm only various forms of mitochondria and plastids are present.

In recent years the morphological changes in the megasporocyte and embryo sac cytoplasm were studied in the light microscope by Eymé (1965, 1966), and in the electron microscope by Mikulska and Rod-

kiewicz (1964, 1967) and Rodkiewicz and Mikulska (1963, 1965, 1966a, b, c). These authors describe very similar structures, but give a different interpretation. Eymé found in the cytoplasm of the investigated cells, beside various systems of endoplasmic reticulum (ER), many plastid forms. On the other hand, in the electron microscope no plastids were seen, only various special systems of endoplasmic reticulum.

Not long ago attention was called to the cytochemical changes accompanying the particular megasporogenesis phases. Changes were mainly investigated occurring in nucleic acid, proteins, lipids, and insoluble polysaccharides (Zinger, 1958; Takao, 1962; Jensen, 1963; Pritchard, 1964; Poddubnaya-Arnoldi, 1964; Alvarez and Sagawa, 1965; Kuran and Marciniak, 1969).

Most of these papers, however, are concerned with only one stage of female gametophyte differentiation, that is the mature embryo sac, and do not treat the occurring phenomenon as a whole.

The purpose of the present study was to investigate by cytochemical methods some of the transformations occurring during megasporogenesis and gametogenesis in *Lilium regale*.

MATERIAL AND METHODS

Embryos from *Lilium regale* flower buds were fixed in Regaud, Champy, Baker and Bouin solutions and in absolute alcohol with glacial acetic acid (3:1, Carnoy). To detect protein and lipids, material was fixed for 18—24 h at 0—4° C in Baker's solution with 7.5 per cent sucrose added was used. The fixed embryos were embedded in paraffin and 3 μ m microtome sections were prepared from younger and 5 μ m sections from older stages. Proteins were identified by bromophenol blue reaction (Mazia et al., 1953) and the ninhydrin-Schiff method (Yasuma and Ichikawa, after Jensen, 1962).

The material for lipid analysis was postchromized in a dichromate-calcium mixture according to Baker (Pearse, 1962). Lipids were visualized by staining with Sudan III and IV (Kay and Whitehead, Pearse, 1962), and Sudan Black B (McManus, after Pearse, 1962). As control served nonpostchromized preparations extracted with pyridine according to Baker (Pearse, 1962). Neutral lipids were detected by Nile Blue reaction after Cain (Pearse, 1962). PAS test for insoluble polysaccharides was made on material fixed in alcohol with acetic acid (3:1) for 24 h according to Hotchkiss, Pearse (1962). The reaction was inhibited by acetylation according to Gersh (Pearse, 1962). Nucleic acid were revealed by staining the preparations with Unna mixture, and DNA was detected by the Feulgen reaction.

Plate I

Megasporocytes in early meiotic prophase. Parallel endoplasmic reticulum systems are visible. Fig. 1 — $\times 1300$, Fig. 2 — $\times 1400$

Plate II

Megasporocytes in early meiotic prophase. Different distribution of parallel endoplasmic reticulum systems is seen. Figs 3, 4, 5, 7 and 9 — $\times 400$, Figs 1, 2, 6 and 8 — $\times 500$

Plate III

Figs 1—6. Megasporocyte in advanced meiotic prophase I, Figs 7, 8. Meiotic division I, Fig. 9. Primary 4-nucleate stage, Steps of cytoplasmic bodies formation, Figs 1, 7, 8 and 9 $\times 400$, Figs 2—6 — $\times 700$

RESULTS

Megasporocyte

All cells in the young embryo divide mitotically, and at first orthotropic embryo transforms to an anatropic one. During this process the nucellus, the megasporocyte and two integuments form. In the subepidermal layer of the orthotropic embryo the archaespore cell begins to differentiate. It is oval in shape and localized in the cell layer under the epiderm. With growth it takes the form of a cone oriented with its base towards the epiderm and it elongates along the long axis of the embryo (Plate II, Figs 1—9). The apex of this cone gradually penetrates into the cell mass. In contrast to the surrounding cells, the archaespore does not show any mitotic activity, it only continuously increases its volume. This growth is proportional to the nucleus growth, but above all, the diameter of the nucleus increases. Karyoplasm is optically much less dense than the cytoplasm, and except of the chromatin filaments and nucleolus, completely transparent. The cytoplasm of such early-prophase cells does not differ from that of the surrounding ones. It is dense with small clear areas, probably minute vacuoles. This cytoplasm contains tiny grains staining with haematoxylin, visible after fixation in Champy and Baker's solutions, and not revealed after fixation in Bouin and Carnoy's solutions. These grains most probably represent mitochondria, spherosomes and proplastids.

The young archaespore cytoplasm exhibits high affinity to Sudan dyes, staining homogeneously with them. On the other hand, in the cells of the chalazal part of the nucellus (on the same preparation) only few sudanophilic grains are seen.

In the cytoplasm of older archaespore cells (slightly elongated on longitudinal section with an almost triangular shape) numerous ergastoplasmic structures are visible. They appear in the form of parallel filaments which actually are cisterns of endoplasmic reticulum (Plate I, Figs 1—2 and Plate II, Figs 1—9). These cisterns are arranged parallelly to one another. At first they lie between the nucleus and the micropylar wall of the archaespore (Plate II, Fig. 2), later they occupy the entire cytoplasm. With growth of the archaespore cell this distribution changes and the endoplasmic reticulum cisterns assume a position perpendicular to the nucleus surface (Plate I, Fig. 1). In the micropylar part these elements of ER are more numerous. The arrangement of endoplasmic reticulum cisterns may vary, some groups are arranged parallelly to the nucleus (Plate II, Fig. 2), others are diagonal (Plate I, Fig. 2 and Plate II, Figs 3—8). The endoplasmic reticulum groups may be separated from one another by regions of granular cytoplasm. In the phase discussed larger accumulation of endoplasmic reticulum is noted in the micropylar part of the cell. The

chalazal part may be free of these systems (Plate II, Fig. 2) or may contain much less of them (Plate II, Figs 4 and 5).

In the period when parallel endoplasmic reticulum systems are observed there appear small and not numerous vacuoles (Plate I, Fig. 2 and Plate II, Figs 3—5). These vacuoles are flattened and seem to be appressed to the endoplasmic reticulum cisterns. Beside cells with small vacuoles, some without any may be found, but with the same kind of endoplasmic reticulum systems. Thus, it would seem that these vacuoles are ephemeric. They probably appear and disappear within a short time period.

During leptotene the number of the endoplasmic reticulum elements visible in the light microscope decreases, and they are no longer parallelly arranged to one another. Sometimes they may be disposed fan-like from the nucleus towards the micropylar pole (Plate II, Fig. 8) or a similar small system may be found at the chalazal pole (Plate II, Fig. 7). Towards the end of leptotene the parallel endoplasmic reticulum systems are no more visible in the light microscope. At this stage of prophase I there occur in the meiocyte only single elements of endoplasmic reticulum irregularly distributed within the cell. In the chalazal part of the perinuclear cytoplasm, however, spherical bodies appear with a distinctly circumscribed dark rim and a lighter centre (Plate II, Fig. 9).

At this stage the nucleolus undergoes strong deformation, it becomes flat and clear spaces appear in it. This coincides in time with a considerable rise of affinity to pyronin of the cytoplasm after staining of the cells with Unna mixture.

The cytoplasm of leptotene meiocytes contains minute sudanophilic grains. After reaction with Nile blue they are pink in colour, this indicates the neutral character of these lipids. The number of these granules increases at the moment when the parallel endoplasmic reticulum systems disappear, moreover, they are disposed around the nucleus and close to the cell walls.

After the reaction for protein, the meiocyte cytoplasm stains homogeneously with the exception of the site where the parallel cytoplasmic reticulum systems exhibiting a much stronger reaction are present.

In the cytoplasm of the meiocyte, being in the zygotene phase, there appears a new category of cytoplasmic bodies. On preparations fixed according to Baker and Champy and then stained with haematoxylin or dyes revealing protein, these structures are most pronounced in the form of coloured spheres not very regular in outline. After additional staining of the lipids with Sudan dyes some of these structures stain uniformly, while in others sudanophilic grains are visible (Plate III, Fig. 2). These structures referred below as cytoplasmic bodies lie compactly in the meiocyte cytoplasm, particularly in its micropylar part.

It is seen in the cytoplasm of pachytene and early diplotene meiocytes that the cytoplasmic bodies consist of several layers giving a positive

reaction for protein (Plate III, Figs 3—5). Sometimes inside these spherical bodies lipid inclusions may be found (Plate III, Figs 4 and 6).

During diakinesis these endoplasmic bodies are dispersed in the whole cytoplasm of meiocytes, however they are more numerous in the micropylar part (Plate III, Fig. 1). Noteworthy is the increase of the number of these bodies as compared with the earlier stages. Towards the end of diakinesis, just before metaphase I the meiocyte reaches its maximal size, its cytoplasm is dense without any vacuoles.

The cytoplasm of lily meiocytes does not contain PAS-positive substances. Thus, the cells of the generative line are the only ones within the embryo deprived of starch. The positive PAS reaction indicates the meiocyte wall (Plate V, Figs 2 and 3).

Development of embryo sac

During metaphase I a large number of cytoplasmic bodies varying in size and stainability with Sudans and protein-revealing dyes is seen in the cell cytoplasm (Plate III, Fig. 7). A similar situation is observed in 2- and 4-nucleate primary stages of embryo sac development. The cytoplasm is still dense and contains numerous cytoplasmic bodies (Plate III, Figs 8 and 9). At this stage of embryo sac development during positioning of the nuclei at 1:3 (Plate IV, Fig. 1), a disappearance of the cytoplasmic bodies occurs at the micropylar pole; their place is occupied by basal cytoplasm, and at a later stage a vacuole appears in this part of the cell. At this stage of the embryo sac development the large cytoplasmic bodies staining homogeneously with Sudan disappear while the smaller ones are further intensively stained by this reagent and protein-revealing dyes.

At the beginning of the secondary 4-nucleate stage the cytoplasmic bodies accumulate in the chalazal part and along the side walls of the embryo sac (Plate IV, Figs 2—6). In the micropylar part of the embryo sac the number of these bodies decreases in connection with the above mentioned vacuolization.

On squash preparations hundreds of embryo sacs in secondary 4-nucleate stage could be seen. Among them about 10 per cent of the embryo sacs exhibited no vacuoles. This seems to indicate that the vacuole-free stage lasts 1/10 of the entire duration time of this stage. During vacuolization and maturation of the secondary 4-nucleate stage of embryo sac development the morphology of the cytoplasmic bodies and their stainability, particularly with Sudan dyes, change. Cytoplasmic bodies of various size from rather large to quite small ones appear (Plate IV, Figs 2, 3 and 6). A new category of large bodies now appears, homogeneously staining with Sudan (Plate IV, Figs 5 and 6). A certain number of cytoplasmic bodies do not stain with Sudan dye or only the minute grains within the cytoplasmic bodies absorb the dye (Plate IV, Figs 2 and 3). Sometimes in the large cytoplasmic bodies close to the vacuole sudanophilic granules are localized, and in others minute lighter areas (vacuoles) may be seen.

Similar bodies with a vacuole inside were found also in other parts of the embryo sac (Plate IV, Figs 2 and 3). The cytoplasm between the lower and upper triploid nucleus contains a large number of the above named bodies. The cytoplasmic bodies between the triploid nuclei are minute and of almost even dimensions, whereas in the cytoplasm zone above these nuclei, beside minute also large cytoplasmic bodies may be seen. Towards the end of the secondary 4-nucleate stage of development of the embryo sac the difference in size between the cytoplasmic bodies seems to disappear (Plate IV, Fig. 4).

In the 8-nucleate embryo sac nearly the whole central cell is occupied by a large vacuole, the cytoplasm being mainly concentrated along the walls. Within it cytoplasmic bodies containing lipids are present. At the triploid polar nucleus, after staining with Sudans 1—4 large agglomerations of sudanophilic and homogenous granules are visible (Plate 4, Fig. 1).

The remaining regions of the mature 8-nucleate embryo sac have but seldom been examined in a well fixed state. The difficulty consisted in that it was not possible to establish conditions for simultaneous fixing of all the cells of the mature embryo sac. The synergides and the egg cell exhibit considerable vacuolization. Usually the egg cell is more vacuolized and 4—10 vacuoles can be distinguished in it. The central vacuole has never been observed, although in the egg cells of other angiospermous plant species it has been described (Jensen, 1963). Several minute cytoplasmic bodies were found in the egg cell cytoplasm, giving a positive reaction for protein and lipids.

The antipodes begin to degenerate usually at the moment of their individualization. This process is most advanced in the lower antipode. Relatively best preserved is the antipode lying closest to the central cell. In the cytoplasm of this cell the characteristic endoplasmic bodies do not occur, the cell is strongly vacuolized and sudanophilic. Frequently, irregularly shaped lipid bodies are visible. The general picture of the antipode cytoplasm resembles the known and described cases of lipophanerosis.

In the embryo sac PAS-positive substances were not found up to the 8-nucleate stage except in the wall surrounding the meiocyte (Plate V, Figs 2 and 3) or the maturing embryo sac (Plate V, Figs 4 and 5). A positive PAS reaction is also found only in the antipodes walls and in the egg apparatus walls (Plate V, Fig. 6).

DISCUSSION

The cytoplasm of the young archaespore has the appearance typical for early stages of cell differentiation. It is homogeneous and some few granules are visible in it, which Eymé (1965) identified after application of the Champy fixative and haematoxylin staining as proplastids, leukoplasts and mitochondria. In the present study it was not possible to identify

the proplastids and leukoplasts under the light microscope. Neither do Rodkiewicz and Mikulska (1963, 1965, 1966a, b, c) describe plastids in any megasporogenesis stages in the electron microscope. After fixation in Regaud, Champy and Baker solutions and staining with haematoxylin, only minute lighter areas are seen in the cytoplasm — most probably vacuoles and minute spherical granules, the identification of which is impossible in this kind of observations.

The different systems of endoplasmic reticulum cisterns well visible in the light microscope were examined by Rodkiewicz and Mikulska (1966a, b, c). Over the entire time of growth of the megasporocyte these authors found parallel endoplasmic reticulum strands forming later spherical or ellipsoid coils. Thus the cytoplasmic bodies appearing towards the end of growth of the megasporocyte, observed in the present study, may be interpreted as one of the end phases of coiling of the system.

The coiling of the parallel systems of endoplasmic reticulum during megasporogenesis in angiosperms has been several times described. It was always associated with megasporocyte differentiation of the *Fritillaria* type. As early as 1898 Bouin and Bouin observed in the cytoplasm of megasporocytes of various *Liliaceae* filamentous structures arranged around the nucleus and transforming further to basophilic bodies with a layered structure. The arising of strands (fibrils) in *Fritillaria* megasporocyte plasm coincides, according to Flint and Johansen (1958), with nucleolar activity, and the filaments formed coil up around the nucleus owing to its rotatory movement.

According to Eymé (1965, 1966), at the end of prophase I there occurs degradation of the organized ergastoplasm and structures form, named by the author cytoplasmic nodules (nodules cytoplasmatiques) which are cytoplasm breakdown products.

The transformation of the microscopic structure of the cytoplasm at the time of transition of the archaespore to the stage of meiosis described here may be compared with the observations in the electron microscope (Rodkiewicz and Mikulska, 1966b). It results from these findings that the ergastoplasmic structures, their parallel systems and the spherical bodies consist of cisterns of endoplasmic reticulum. Some cases may be quoted when the endoplasmic reticulum systems could be seen in the light microscope (Eymé, 1965, 1966; Kuran and Marciniak, 1969). It may be assumed that the structures described in these cases were actually cisterns or groups of endoplasmic reticulum cisterns. The parallel systems are better visualized after treatment with Carnoy's fixative than after fixation in Regaud, Champy or Baker's solutions. It would seem therefore, that in the case of drastic protein denaturation with acetic acid and ethanol, the ultrastructural cisterns coalesce and become better visible in the light microscope. These pictures although they

are to some degree artefacts, reveal nevertheless the true endoplasmic reticulum system in the meiocyte.

Megasporocytes with the strongly developed system of endoplasmic reticulum exhibit a high affinity for pyronin after staining with Unna mixture, this indicating an abundant ribonucleic acids contents during meiocyte development may be evidence of the presence of numerous ribosomes.

The volume of the lily megasporocyte increases within several days about a dozen times or so. All this time the cytoplasm remains dense and nonvacuolised (minute ephemerical vacuoles appear for only a very short period). Thus, the volume increment does not result from ordinary hydration but is due to synthesis of cytoplasm components. The increase in cytoplasm mass ceases at the end of prophase, thus, protein synthesis lasts over prophase I of meiosis. This observation agrees with the results of investigations on microsporogenesis in *Tulipa* (Hotta and Stern, 1963, 1965). It was, namely, found that during prophase I RNA and protein synthesis do not cease, the peak for RNA falling to the period between pachytene and diplotene, thereafter a drastic fall occurs. Treatment at this time with purine analogues (Stern and Hotta, 1963) causes synthesis of defective RNA which produces various disturbances in the course of meiosis.

The time coincidence during prophase I of depletion of the nucleolar substance in the *Lilium regale* megasporocyte and of the disorganization of the endoplasmic reticulum cistern systems may be referred to the above quoted decrease in RNA and protein synthesis in the end of prophase I.

The megasporocyte reaches its largest dimensions in the period of prometaphase I. Its cytoplasm is dense and filled with cytoplasmic bodies. At this time the dry mass of the cell cytoplasm measured in the interference microscope is the same as in the primary 4-nucleate stage (Kuran, 1968). This fact agrees with cytological observations. It seems that the number and size of cytoplasmic bodies do not increase after prometaphase I up to the primary 4-nucleate stage of the embryo sac development.

The appearance of minute irregular cytoplasmic and vacuolized bodies and the considerable decrease in sudanophilia of the structures in the secondary 4-nucleate stage is connected with the high activity of such hydrolytic enzymes as acid phosphatase, nonspecific esterase and lipase (Marciniak and Kuran, 1969).

Structures similar to the cytoplasmic bodies observed in lilies are described by Camefort (1962, 1963, 1965) in gymnosperms. In the oosphere of *Ginkgo* he found cytoplasmic bodies which he called "yolk inclusions". These are separated from the basal cytoplasm by two membranes sometimes connected with the endoplasmic reticulum. Their

cytoplasmic, and not paraplasmic character is indicated by the segments of endoplasmic reticulum, mitochondria and Golgi structures which they contain. The same author found in the pine oosphere cytoplasmic inclusions which were not completely separated from the basal cytoplasm. Other cytoplasmic inclusions consisted, according to this author, of a number of concentric cytoplasm layers separated by deformed plastids. During oogenesis in *Pteridium aquilinum* formation of concentric endoplasmic reticulum systems has also been observed (Bell and Mühlethaler, 1962).

Moreover, concentric membrane systems have been revealed in other plants during sporogenesis. In the embryo sac of *Linum usitatissimum* they may, according to Vazart and Vazart (1967), play some role in cell nutrition. Jensen (1965) also suggests that the occurrence of concentric endoplasmic reticulum coils in the central cell of *Gossypium* is connected with the metabolic activity of this cell.

Coiling of endoplasmic reticulum elements under the influence of mechanical and chemical factors has also been noted in somatic cells. These changes receded as a rule after transfer of the plant to normal conditions (Whaley et al., 1964; Gavoudan et al. 1960; Wrischer, 1960; Dexheimer, 1966a, b).

In the course of maturation of the 8-nucleate embryo sac, large parts of the cytoplasm and whole cells undergo lysis and vacuolization, particularly in the antipodes and central cell. The cytoplasm remaining in the central cell contains only few cytoplasmic bodies and sudanophilic structures which undergo further hydrolysis.

The function of the cytoplasmic bodies consists in isolation of certain cytoplasm areas in early period of embryo sac differentiation, particularly in the megasporeocyte. The isolated cytoplasm may once more merge with the surrounding basal cytoplasm after decomposition of the surrounding membranes. In view of the activity of hydrolytic enzymes revealed in the regions of the embryo sac where the cytoplasmic bodies degenerate (Marciniak and Kuran, 1969), it would seem that these bodies are completely hydrolyzed and the hydrolysis products are introduced into the metabolism of the maturing embryo sac. In this connection the cytoplasmic bodies should be considered as structures associated to some extent with nutrition, and the processes of their disintegration, disorganization and hydrolysis towards the end of embryo sac differentiation immediately precede and prepare fertilization.

The lipid droplets in all stages gave a pink colouring with Nile blue, this indicating their neutral character. They may, therefore, be considered as storage material.

The same would apply also to lipids from breakdown of lipoproteins complexes. The process of decomposition of these complexes may occur during enzymatic digestion of the endoplasmic reticulum, both during

coiling of the parallel membrane systems to cytoplasmic bodies and during digestion of the bodies at a later period of embryo sac development.

Information is scarce as regards the site of synthesis of storage lipids. It is difficult to establish by which cellular components they are synthesized. In many plants they may be formed in the plastids, but the main sites of lipid production are the endoplasmic reticulum, spherosomes and cytoplasmic inclusions.

In the megasporocyte and embryo sac of lilies a part of the lipids may be derived from breakdown of endoplasmic reticulum membranes, although it is more probable that at least part of these lipids or their substrates originate from the cells surrounding the embryo sac. This is supported by the occurrence of numerous plasmodesmata in the chalazal wall of the embryo sac (Rodkiewicz and Mikulska, 1966a).

Noteworthy is the earlier appearance of lipid droplets in the chalazal part of the nucellus than in the megasporocyte. This succession of events may suggest that the megasporocyte and embryo sac lipids pass from the chalazal part of the nucellus.

Electron microscopic data do not conform completely with light microscope results. After fixation with osmium tetroxide no such large lipid bodies are found in the megasporocytes and lily embryo sacs as are seen in light microscope preparations (Rodkiewicz and Mikulska, 1966a, b, c). It is known that fixation with osmic acid well preserves and contrasts lipid substances. It results from electronogram analysis that the visible osmiophilic bodies are present both in protein substances and in the basal cytoplasm. These protein bodies are usually built of concentrically arranged endoplasmic reticulum cisterns and cytoplasm contained between them. Frequently it may be seen that the cytoplasm in the particular layers and in the middle of the system is not uniformly osmiophilic. Moreover, these systems are not ideally concentric. The more osmiophilic cytoplasm probably also stains stronger with Sudan. Thus, such an area after reaction with Sudan may have the appearance of a lipid droplet, although in the electron microscope it is an area of dense cytoplasm. It is not excluded of course that the lipids are washed out during electron microscopic preparation, there also exists another danger — the merging of minute lipid droplets during fixation for the light microscope. This merging of lipid droplets present in the cytoplasmic bodies into large ones is not very probable in view of the separation by endoplasmic reticulum membranes of these bodies into a series of zones and their separation from basal cytoplasm. It cannot be ruled out, on the other hand, that the lipid droplets in the basal cytoplasm may combine into larger spherical bodies.

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Badania cytochemiczne megasporocytu i woreczka zalążkowego Lilium regale w różnych fazach rozwoju

Streszczenie

Celem niniejszej pracy było zbadanie metodami cytochemicznymi niektórych przemian zachodzących podczas gametogenezy w komórkach żeńskiej linii generatywnej u *Lilium regale*.

Podczas rozwoju woreczka zalążkowego *Lilium regale* występują cztery fazy strukturalnej organizacji cytoplazmy:

1. Faza bez widocznych w mikroskopie świetlnym struktur endoplazmatycznego retikulum (młoda archespora).
2. Faza występowania równoległych układów endoplazmatycznego retikulum (wczesna profaza do leptotenu).
2. Faza zwijania się równoległych układów endoplazmatycznego retikulum w ciała cytoplazmatyczne (od leptotenu do diakinezy).
4. Faza rozpadu ciał cytoplazmatycznych (stadium czterojądrowe wtórne i stadium ośmiojądrowe rozwoju woreczka zalążkowego).

W prometafazie pierwszego podziału mejotycznego ciała cytoplazmatyczne zajmują prawie całą przestrzeń mejocytu. Występują w nich różnej wielkości inkluzje lipidowe. Sytuacja taka utrzymuje się w dwujądrowym i czterojądrowym pierwotnym stadium rozwoju woreczka zalążkowego. W stadium czterojądrowym wtórnym zmienia się morfologia i chemizm ciał cytoplazmatycznych. Podczas dojrzewania woreczka zalążkowego cytoplazma ulega częściowej hydrolizie. Szczególnie proces ten zaznacza się na biegunie chalazalnym woreczka zalążkowego. Należy sądzić, że produkty hydrolizy zostały włączone w metabolizm dojrzewającego woreczka zalążkowego. Tak więc ciała cytoplazmatyczne i inkluzje lipidowe syntetyzowane podczas pierwszej profazy mejotycznej stanowią materiał zapasowy zużywany podczas dojrzewania woreczka zalążkowego *Lilium regale*.