EVENTS IN THE CYTOPLASM DURING MALE MEIOSIS IN LILIUM

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SUMMARY

An electron microscopic investigation of the events associated with meiosis in Lilium has revealed a number of changes in both the organelar population and the other cytoplasmic components. Ribosome numbers decrease significantly in early prophase and are later replenished in the tetrads, a process most likely involving the newly arising cytoplasmic nucleoloids. The organelles show a cycle of de- and redifferentiation and later in meiosis unusual internal structures can be seen before these organelles enter a division phase resulting in increased numbers. The localization of acid phosphatase during these changes has also been studied using electron microscopic cytochemical methods. In early prophase, considerable amounts of acid phosphatase are found in vesicles scattered through the cytoplasm; activity is also found in association with most membranous surfaces and often markedly associated with condensing mitochondria. Later in prophase the enzyme activity decreases to normal levels. Electron microscopic autoradiography revealed that DNA is synthesized in both plastids and mitochondria during meiotic prophase with activity reaching a peak during zygotene and ceasing by diakinesis and tetrad formation.

These changes point to a certain independence of organelles from nuclear control during meiosis. The events are also evaluated in relation to a cytoplasmic clearing mechanism, which may occur in preparation for the changeover from sporophytic to gametophytic control and the development of gametes.

INTRODUCTION

Since the work of Guillermond (1924) cytoplasmic changes have been known to accompany male meiosis in flowering plants. These events have now been shown to include a cycle of RNA metabolism (Mackenzie, Heslop-Harrison & Dickinson, 1967; Dickinson & Heslop-Harrison, 1970a) and the dedifferentiation of the organelle population (Dickinson & Heslop-Harrison, 1970b). In some species this dedifferentiation is so complete that some authors concluded that the organelle line is discontinued at this point (Bal & De, 1962), but the use of improved methods of preparation for the electron microscope proved this not to be the case (Marumaya, 1968). There is little doubt, however, that during meiosis in lower plants (Pettitt, 1978) — and in female meiosis of higher plants (Dickinson & Potter, 1978) — a proportion of the organelles can be eliminated. This elimination has yet to be examined in detail and remains to be demonstrated in male cells. The chemistry of these events is, however, completely unknown. Vaughn, DeBonte, Wilson & Schaffer (1980) have described a further phase of organelle elimination to take place during maturation of

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the male gametophyte, which results in the inheritance of maternal organelles only. However, since biparental inheritance of organelles occurs in many species (Russell, 1980; Sears, 1980) this event cannot be common to all plants, and is unlikely to be related to the earlier 'meiotic' dedifferentiation.

The dedifferentiation of the organelles in prophase coincides with a striking drop in levels of cytoplasmic RNA and it has been suggested that the RNA lost includes species involved in the control of organelle development (Dickinson, 1981). Such changes as are observed might thus be anticipated in organelles freed from nuclear control. The relationship between the nucleus and organelles at this point must therefore be understood before any conclusion as to the significance of these events can be reached. It is known that the nucleus synthesizes both RNA (Williams, Heslop-Harrison & Dickinson, 1973; Porter, Bird & Dickinson, 1982) and DNA (Hotta, Ito & Stern 1966; Riley & Bennett, 1971; Godward & Peristianus, 1976; Porter et al. 1982) in meiotic prophase and much of this RNA finds its way into the post-meiotic cytoplasm (Williams et al. 1973). However, nothing is known of its information content, although it has recently been suggested that the organelles themselves may synthesize DNA during meiotic prophase (Smyth & Shaw, 1979). Since many of the investigations into DNA synthesis in meiocytes have assumed that all synthesis takes place in the nucleus, it is important to discover whether organelles do in fact synthesize DNA and, if so, what proportion of the total meiocyte synthesis is accounted for by organellar activities.

We have used electron microscopic cytochemistry and autoradiography to examine the continuity of organelles during male meiosis in *Lilium*, and to establish whether these organelles are involved in the metabolism of nucleic acids. The results from this work are discussed in terms of current views of the alternation of generations, the formation of gametes and the autonomy of organelles.

**MATERIALS AND METHODS**

Electron microscopy of *Lilium* meiocytes was carried out as described by Dickinson (1971). The distribution of acid phosphatase within the tissues was investigated using a derivation of the Gomori technique, as modified by Stewart & Pitt (1977). In this method, staged filaments of meiocytes were obtained as described by Porter et al. (1982), and fixed for 2-5 h in ice-cold 3 % glutaric dialdehyde,

Fig. 1. Two premeiotic pollen mother cells showing starch-containing plastids (p) normal mitochondria (m) and high levels of ribosomes (r). × 9280.

Fig. 2. Meiocyte during early lepotetene. The plastids (p) are dividing and starch (s) is undergoing digestion. High levels of lipid (l) are present in this cytoplasm. The mitochondria (m) appear normal. × 22 780.

Fig. 3. Early-zygotene meiocyte showing condensed mitochondria (m) and bodies, demonstrated elsewhere, to contain acid phosphatase (a). Fewer ribosomes are present. × 12 460.

Fig. 4. As Fig. 3, but showing higher levels of ribosomes in the tapetum (t). Lipid droplets (l), dedifferentiated plastids (p) and enzyme bodies (a) are visible. × 3620.

Fig. 5. As Fig. 3, but at a higher magnification. Legend as Fig. 4. Ribosomes are at their lowest levels at this stage. × 16 320.
Male meiosis in Lilium

Figs 1–5
buffered to pH 7.2 in 0.03 M-PIPES. The cells were then washed in several changes of ice-cold 0.05 M-acetate buffer (pH 5.1) containing 5% sucrose over a period of 4 h. Following this period of pH acclimatization, these filaments were incubated for 30 min at room temperature in 10 ml of 0.05 M-acetate buffer (pH 5.1) containing 0.001 M-sodium β-glycerophosphate, 0.004 M-lead nitrate and 5% sucrose. Two controls were generally run, one containing 0.01 M-sodium fluoride, the other omitting the substrate (see Fig. 6). Incubated cells were washed in three changes of ice-cold PIPES buffer (pH 7.2) containing 5% sucrose over 2 h, and post-fixed in a 1% aqueous solution of osmium tetroxide for 3 h. Finally, the filaments were washed twice in distilled water and prepared for electron microscopy in the usual manner.

The incorporation of [3H]thymidine and [3H]uridine into meiocyte filaments of Lilium cinnabar and the radioautographic methods used to localize the radiotracer in the electron microscope were as described by Porter et al. (1982).

RESULTS

The data from the cytochemical and autoradiographic experiments are presented in very different manners and for this reason they are considered under separate headings.

Structural and cytochemical events accompanying meiosis

The cytology of premeiotic pollen mother cells of Lilium is that of a normal plant meristematic cell (see Fig. 1). Many plastids are present, generally containing starch, and the mitochondria are of orthodox morphology. The cells contain high levels of ribosomes, and some are bound to the membranes of endoplasmic reticulum. As the cells enter preleptotene the starch content of the plastids begins to decrease and the organelles themselves become conspicuously pleiomorphic (see Fig. 2). Also at this time lipid begins to accumulate in the cytoplasm, generally in the form of spherical

Figs 6–12 depict material treated to reveal acid phosphatase.

Fig. 6. ‘Control’ enzyme preparation, minus substrate. The material has not been post-stained but the principal features of the cytoplasm are visible. There is a very slight deposit of electron-opaque material, but it is not localized. × 14 750.

Fig. 7. Reaction product (arrows) in an enzyme body. Slight deposit also occurs in the general cytoplasm of this cell, which is in early prophase. × 42 000.

Fig. 8. Meiocyte cytoplasm during early zygotene showing heavy deposition of reaction product over the cytoplasm. Particularly large accumulations are present within an enzyme body (a). × 13 125.

Fig. 9. Detail of cytoplasm during late zygotene. Reaction product is present associated with individual clusters of ribosomes (r). Material post-stained with lead citrate. × 172 350.

Fig. 10. Material as in Fig. 9, but unstained and showing a multimembraned inclusion. Reaction product is deposited over the investing membranes (arrows), a section of endoplasmic reticulum (er) and mitochondria (m) both outside and within this inclusion. × 27 300.

Fig. 11. Low-power micrograph of meiocyte at pachytene. Deposition of reaction product over the chromatin (arrows) is clearly demonstrated. The nucleolus (no) remains unaffected. × 6850.

Fig. 12. Deposition of reaction product on mitochondria (arrows) during early pachytene. One organelle (o) appears to be degraded. The inset shows high levels of reaction product in a mitochondrion. × 29 800; inset, × 48 250.
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Figs 6–12
Figs 13–18
Male meiosis in Lilium

By the leptotene stage the majority of the plastids are free from starch and many display profiles indicating division. The morphology of the mitochondria also alters in leptotene, these organelles taking on a highly condensed aspect (see Fig. 3), being reduced in size from some 1.5 μm to 0.5 μm in diameter. Division profiles are also present. Towards the end of the leptotene stage and at the onset of zygotene the numbers of ribosomes within the meiocytes become strikingly reduced (see Figs 4, 5). This phenomenon is well-documented in the literature (see Dickinson & Heslop-Harrison, 1977, for a review) and needs no further elaboration.

Acid phosphatase may first be detected in significant quantities in premeiotic cells. Here, small amounts of the enzyme are associated with the mitochondria, as is the case throughout all stages (e.g. see Fig. 12), but by far the major part of the reaction product is contained within spherical vesicles measuring some 0.2 μm in diameter (see Fig. 7). The frequency of these phosphatase-rich vesicles rises to a peak in leptotene, when about 10 may be distinguished within a single transversely sectioned meiocyte. Also during this stage the enzyme becomes far more widely dispersed throughout the cytoplasm, being associated with most membranous surfaces. Some reaction product occurs free in the cytoplasm (see Fig. 8); indeed, on occasion it may be seen in association with individual ribosomes (see Fig. 9). Where the cisternae of the endoplasmic reticulum are arranged in concentric spheres – forming a multio-membraned inclusion – acid phosphatase is presented in both inner and outer layers of membrane (see Fig. 10). At all stages of development considerable quantities of reaction product are deposited within the chromatin (see Fig. 11). This deposition is so well-localized that it may be seen to be absent from the lateral elements of the synaptonemal complex in the pachytene chromatin.

By late zygotene the cytoplasm appears considerably dedifferentiated. Internal structure is largely lost from the plastids, which now exist solely as double-membraned inclusions containing between one and ten small osmiophilic globules and small membranous cisternae. Ribosomes are all but absent and profiles occur, indicating that some mitochondria contain extraordinarily large amounts of acid.

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Fig. 13. Mitochondrion (m) and plastids (p) in the cytoplasm of the young tetrad. The plastids contain an association between plastids and membrane (mpa), while the mitochondrion contains electron-opaque material (eom). × 19 280.

Fig. 14. Cytoplasmic nucleoloid (n) in the tetrad cytoplasm. Large numbers of ribosomes (arrows) appear associated with the nucleoid surface. × 31 150.

Fig. 15. Detail of condensed mitochondria (m) containing electron-opaque material (arrows) in the cytoplasm of young microspores just prior to their release from the tetrad. Ribosome levels are returning to normal. × 46 750.

Fig. 16. Mitochondria (m) displaying a more conventional profile in young microspores following release from the tetrad. The low levels of ribosomes result from a rapid expansion of the cell. × 60 230.

Fig. 17. Early binucleate pollen grain. The vegetative cell (v) contains amyloplasts (p) and mitochondria (m), while the generative cell (g) contains only mitochondria. × 8 230.

Fig. 18. Autoradiograph of meiocyte at late zygotene following feeding with [3H]thymidine. Note the silver grains (arrows) associated with the plastids (p). × 15 180.
phosphatase (see Fig. 12, inset) generally located in the cristae. A small number of these organelles also appears to degenerate (see Fig. 12).

From zygotene of prophase to metaphase I very little cytoplasmic differentiation takes place. The general distribution of acid phosphatase throughout the cytoplasm declines rapidly at this time, returning to the levels detected in premeiotic cells. The mitochondria remain condensed, and the double- and multiple-membraned inclusions (DMI and MMI) persist. In late prophase the plastids may develop a conspicuous inclusion, known as the membrane–particle association (MPA) (see Fig. 13). Details of this structure and of plastid differentiation at these stages are well-recorded in the literature (Dickinson & Heslop-Harrison, 1971; Dickinson, 1981) and are not considered here. Cursory observation of organelle frequency over this period suggests that the numbers of both plastids and mitochondria may fall slightly.

During cytokinesis the phosphatase-rich vesicles disappear from the cells and a new class of inclusions arises. These are the electron-opaque cytoplasmic nucleoids, which are abandoned at the ‘metaphase plates’ during anaphase I and II (see Fig. 14). They measure up to 0.8 µm in diameter and their structure, fate and rôle in the restoration of ribosomal levels are discussed at length by Williams et al. (1973). It is in the tetrad of young microspores that the MPAs of the plastids become most evident, and the mitochondria begin to accumulate electron-opaque material in their matrices (see Figs 13, 15). These aggregates, which are at least 10 nm in size, appear granofibrillar and may occur in mitochondria seen in a single section. They remain as a mass within the matrix, and are not penetrated by the internal lamellar system.

Towards the end of the tetrad stage, division profiles are frequently observed in both plastids and mitochondria, and the populations of both these organelles increase markedly. Careful observation reveals that the MPA divides prior to plastid division; also, when the mitochondria divide each daughter organelle contains an electron-opaque accumulation. The tetrad cytoplasm appears normal in its ribosome population and in the distribution of acid phosphatase, except in the regions where the pollen wall is being synthesized.

Conventional structure returns to both plastids (see Fig. 7) and mitochondria (see Figs 16, 17) following break-up of the tetrad. The MPAs and accumulations of electron-opaque material are lost from these organelles as they redifferentiate. The young spore is also the last stage at which the DMI and MMIs are visible and it is noteworthy that now they appear to contain high levels of acid phosphatase both in their lumens and on their investing membranes.

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Fig. 19. Autoradiograph of pollen mother cell at late pachytene following labelling with [³H]thymidine. Silver grains (arrows) are present associated with plastids (p), mitochondria (m) and chromatin (c). × 7000.

Fig. 20. Several silver grains (arrows) associated with a dividing plastid (p) in the cytoplasm of a meiocyte at late zygotene, previously fed with [³H]thymidine. × 29 270.

Fig. 21. Autoradiograph of early pachytene cytoplasm of a meiocyte previously labelled with [³H]thymidine. One silver grain (arrows) is associated with a mitochondria (m) while the other is situated over ground cytoplasm. × 49 520.
Male meiosis in Lilium

Figs 19–21
Meiotic stages

Fig. 22
Male meiosis in Lilium

[3H]thymidine incorporation into the meiocyte cytoplasm

A series of electron microscopic autoradiographs showing the incorporation of [3H]thymidine by Lilium meiocytes is shown in Figs 18–21. A large number of autoradiographs were subjected to statistical analysis and the relative rates of incorporation of [3H]thymidine by plastids, mitochondria and cytoplasm can be seen in Fig. 22, where the data are plotted together with 95% confidence limits. The calculation of relative rate takes into account the areas occupied by the various components within the cell, and a value of 1.0 represents a random distribution of grains. The time scale is broken on the abscissa to indicate the longer duration of the development from division II to tetrad relative to the stages of meiotic prophase.

The plastids and mitochondria show a progressive increase in [3H]thymidine incorporation during early meiotic prophase, which attains a peak during zygotene as shown by an increase in the number of grains associated with both classes of organelles (Fig. 22A, C). In many cases more than one grain is seen in association with a particular plastid (Fig. 18), and label is frequently seen in association with dividing organelles (Fig. 20). Incorporation of label into organelles declines through pachytene, for fewer silver grains are present (Fig. 19). Of those remaining, however, most are associated with organelles. [3H]thymidine grains show no significant association with the DMIs or MMIs, except in cases where they enclose organelles. By diakinesis, organelar incorporation of label had declined to a very low level and remained so through to the tetrad stage (Fig. 22A, C). The cytoplasm shows a background incorporation of [3H]thymidine with little variation throughout meiosis (Fig. 22E).

The analysis of the data may be subject to certain errors that could affect estimations of mitochondrial activity. Such errors may, particularly, be caused by their small size relative to that of grains generated by [3H]thymidine (0.5 μm diam.). This similarity in size means that mitochondria associated with grains could well be obscured by the grains themselves, leading to an underestimation of mitochondrial activity. However, plastids and mitochondria show remarkable similarity in their relative rates of incorporation during meiotic prophase and it is obvious from the data that both classes of organelles are incorporating label at a very high rate in mid-prophase.

[3H]uridine incorporation into the meiocyte cytoplasm

The cytoplasm exhibits a low relative rate of [3H]uridine incorporation throughout meiosis. There are small fluctuations in the relative rates of incorporation of the plastids (Fig. 22B) and mitochondria (Fig. 22D) at zygotene and late pachytene, while the remainder of the cytoplasm shows fairly low activity (Fig. 22F). However, given

Fig. 22. Relative rates of incorporation of [3H]thymidine (A, C, E) and [3H]uridine (B, D, F) by Lilium microsporocytes at different stages of meiosis. PL, preleptotene; EL, early leptotene; L, leptotene; LL, late leptotene; EZ, early zygotene; Z, zygotene; LZ, late zygotene; EP, early pachytene; P, pachytene; LP, late pachytene; D, diplotene; DII, division II; T, tetrad. A, B, plastids; C, D, mitochondria; E, F, cytoplasm.
the variability of the data as indicated by the 95% confidence limits, it is most unlikely that these changes are of any significance.

**DISCUSSION**

*Levels of hydrolytic enzymes in the meiotic cytoplasm*

The cytochemical studies reported here go some way to support earlier work that indicated high levels of hydrolytic enzymes to be characteristic of the cytoplasm during meiotic prophase in some plants (Knox, Dickinson & Heslop-Harrison, 1971). This new data must not, however, be regarded in any way as quantitative, and relates only to the distribution of acid phosphatase within individual cells. The techniques used are inherently susceptible to variability (Stewart & Pitt, 1977) and comparisons— even between individual cells—are probably invalid. Some useful information may nevertheless be provided by these results.

Firstly, some cell components retain a steady level of acid phosphatase activity throughout meiosis. The most conspicuous of these are the chromosomes and the mitochondria. The deposition of reaction product in the chromatin following Gomori treatment is a regular feature (Stewart & Pitt, 1977). It is interesting in this connection that de Jong, Olson & Jansen (1967) have reported that glutaraldehyde stimulates a nuclear acid phosphatase in cells of *Nicotiana tabacum*. Since a considerable spectrum of enzymes will produce reaction product in this assay (Hall & Davie, 1971) it is not surprising that some reaction occurs in the mitochondria. However, these organelles displaying large amounts of activity must differ in some way from the remainder of the population and are discussed at length in a subsequent section.

The large vesicles, rich in acid phosphatase, appear to be characteristic of early prophase. This origin is unclear, but preliminary studies suggest that they are derived from cisternae of the endoplasmic reticulum. Their disappearance coincides with a rapid increase in cytoplasmic free acid phosphatase and it may reasonably be assumed that at least a portion of this enzyme is derived from the vesicles. Since these bodies accumulate and, perhaps, secrete hydrolytic enzymes they most probably should be termed lysosomes.

As meiosis proceeds much of the enzymic activity appears to be lost from the cytoplasm so that, by the dyad stage, little is detectable except in the small vesicles. While this pattern of enzyme activity follows quite closely the cytophotometric data from *Cosmos* (Knox et al. 1971) its significance remains far from clear. It is tempting to associate the presence of an active phosphatase with the low levels of cytoplasmic RNA characteristic of this period, especially as the enzyme may be seen to be intimately associated with ribosomes. Such hydrolytic enzyme activity could have a highly disruptive effect on all types of cytoplasmic RNA although attempts to measure any difference in the levels of RNase have produced highly equivocal results (Porter & Dickinson, unpublished). While all the measurements of RNA levels reported so far (e.g., see Mackenzie et al. 1967) refer presumably to ribosomal RNA, it is interesting to note that preliminary experiments show messenger RNA to be similarly affected at this stage of development (Porter & Dickinson, unpublished).
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On the basis of structural evidence the membranes of the MMIs have been proposed to confer some immunity to hydrolytic enzymes on their contents (Dickinson & Heslop-Harrison, 1977), and it is thus disappointing that reaction product is evenly deposited throughout these structures. Small differences in enzyme activity have been detected within both MMIs and DMIs, and this work continues, using more sensitive methods of enzyme assay. Certainly much information held in the cytoplasm does survive meiosis; for example, the patterning of the complex pollen exine has been shown to be sporophytically determined (Heslop-Harrison, 1971). Further, DMIs, identical to those formed in meiotic prophase have been demonstrated to play a key rôle in the post-meiotic synthesis of the nexine layer of the pollen wall (Dickinson & Heslop-Harrison, 1971).

Despite the high level of accord between the cytochemistry reported here and the cytophotometric data on RNA and phosphatase levels, confirmation that RNA is actively degraded must await a comparison between the rate of removal of RNA during meiotic prophase and the degradation of RNA that accompanies turnover in somatic tissue. Such work is in progress.

Behaviour of organelles during meiosis

The organellar dedifferentiation described during meiosis agrees with many earlier reports in the literature (Bal & De, 1961; Marumaya, 1968; Dickinson & Heslop-Harrison, 1970b). In most plants, mitochondria are reduced to small, spherical 'promitochondria' while the plastids may lose starch reserves, become isodiametric and, in some instances, lose the major part of their structure. In Lilium, where the most detailed studies have been made, starch is lost altogether and the plastids are reduced to large spherical inclusions (Dickinson & Heslop-Harrison, 1971).

The phosphatase-rich mitochondria present in the cytoplasm at this stage are of particular interest for they may represent the first step in the degradation of these organelles. Certainly, degraded mitochondria are frequently seen in the cells and similar profiles have been described both in the maturing egg-cells of ferns (Bell & Mühlethaler, 1962) and in the megaspore mother cells of higher plants (Dickinson & Potter, 1978). Their appearance may signify an elimination of a proportion of the organelles during meiosis. However, the rationale for such a mechanism is not easily understood, unless it constitutes part of a process of cytoplasmic reorganization for subsequent transmission to the next generation.

Organellar dedifferentiation occurs while low levels of RNA persist in the cytoplasm and it has been proposed (Dickinson, 1981) that this may result from the severance of a level of communication between the nucleus and organelles at this stage. At first sight this dedifferentiation might seem to result from simple starvation of the cells, but since energy reserves are changed from starch to lipid and ribosomes are also lost from plastids, such an inference is untenable – especially when considered in the light of the very specialized redifferentiation that follows these events (Dickinson, 1981).

The metabolism of nucleic acids by organelles also shows that they are far from inactive during the stages of dedifferentiation. At the start of meiotic prophase there
is a period of low activity with very little DNA or RNA synthesis but this is followed by a massive synthesis of DNA. Smyth & Shaw (1979) first provided evidence of meiotic cytoplasmic nucleic acid synthesis, and from our data and those of others (Smyth, personal communication) it is obvious that the majority of this synthesis takes place in the plastids and mitochondria. Using physiological methods, Stern and co-workers have revealed phases of DNA synthesis during meiotic prophase (Hotta & Stern, 1971), which are always presumed to occur within the nucleus. Simple inspection of autoradiographs shows that the cytoplasmic incorporation of precursor constitutes a sizeable proportion of total cellular uptake. However, the methods used in Stern's studies (Hotta et al. 1966; Hotta & Stern, 1971) would have revealed only incorporation into nuclear DNA, and indeed our own statistical analysis of nuclear-associated label (Porter et al. 1982) points to a peak of DNA synthesis in zygotene and pachytene.

Redifferentiation of organelles is clearly accompanied or preceded by a phase of organellar DNA synthesis. In *Lilium*, the redifferentiation of both plastids and mitochondria also seems to involve the appearance of unusual inclusions. The MPA of the plastids is discussed at length elsewhere (Dickinson & Heslop-Harrison, 1970b; Dickinson, 1981) and the electron-opaque accumulations have also been reported as occurring in female tissue (Dickinson & Potter, 1978). Both the MPA and the electron-opaque material have been shown, by enzymic digestion, to be rich in protein and to contain RNA. If, indeed, organellar dedifferentiation results from a breakdown in the chain of command from the nucleus, the appearance of these unusual inclusions during redifferentiation may signify the resumption of control, in the form of new RNA and protein synthesized within the organelle. Also, when MPAs are observed in plastids undergoing division, they appear to be intricately involved in the process, sometimes behaving like a plastid 'nucleus' dividing in step with the organelle (Dickinson, 1981). Unfortunately, the behaviour of organelles during meiosis is not consistent for all higher plants. In *Cosmos bipinnatus*, for example, inclusions are seen in both plastids and mitochondria but, in the tetrad stage, the mitochondria undergo a phase of association with the nucleus (Dickinson, 1979). Clearly, until events directly linked with meiosis can be dissociated from changes that form part of the alternation of generations and gametogenesis, no firm conclusions may be drawn as to their significance.

*The significance of the cytoplasmic events that accompany meiosis*

The evidence presented here confirms that meiotic prophase in *Lilium* is accompanied by a conspicuous but ordered dedifferentiation of the meiocyte cytoplasm. The levels of cytoplasmic RNA fall very rapidly, either actively removed by specific hydrolytic enzymes, or declining due to a cessation of synthesis and the operation of normal breakdown processes. The new RNA that populates the cytoplasm in subsequent stages is derived from the cytoplasmic nucleoloids (Williams et al. 1973) and clearly is a product of the new haploid nucleus. Preliminary experiments suggest that these nucleoloids contain ribosomal RNA, and thus this 'cycle' emerges as a mechanism by which new 'postmeiotic' information can be expressed...
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in a cytoplasm from which previous information-carrying molecules have been erased.

It is tempting to propose that this rationalization of the cytoplasm also includes the organelles, for it is clear that they undergo considerable dedifferentiation and that some of their number are eliminated. If so, a mechanism would have to exist whereby some organelles were selected for transmission while others were eliminated. There is currently no evidence for such a mechanism, except perhaps the postmeiotic association of mitochondria with the nucleus in Cosmos (Dickinson, 1979), a phenomenon also reported to occur during animal gametogenesis (Baker & Franchi, 1969). Thus, all that may reasonably be concluded is that fundamental changes occur in the organelles of meiocytes, some of which may result from short-term changes in the relationship between the nucleus and the cytoplasm.

Gametogenesis, meiosis and alternation of generation take place over a very short period in higher plants, and so the cytoplasmic 'cycle' may not be linked specifically with meiosis. Information from other plants in which these processes are separated is, however, conflicting. In ferns, where meiosis is followed by sporogenesis as in the angiosperms, changes in cytoplasmic RNA have been reported (Sheffield & Bell, 1979), while in Chlamydomonas these events apparently occur during gametogenesis, quite apart from meiosis (Siersma & Chiang, 1971). Perhaps the only factor common to these processes during which the RNA cycle takes place is that they are involved in the formation of cells that give rise to new individuals. The removal of this RNA might thus ensure that the cytoplasm transmitted to the next generation is more closely aligned to the information carried in the nucleus and better fitted for the rapid differentiation that is to follow.

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